ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400332 Year: 98 Project Number: 1265-12320-001-00 D
Mode Code: 1265-20-00 STP Codes: 1.2.2.4 60% 3.2.2.5 40%
NATL PROG(S) 102 Animal Production Systems 40%
201 Water Quality & Management 60%

Title: ASSESSMENT OF AGRICULTURAL VS NATURAL HABITATS AS SOURCES OF CRYPTOSPORIDIUM PARVUM

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Cryptosporidiosis is an emerging parasitic disease causing diarrheal illness and death in livestock and humans worldwide. Although eight named species are considered valid, infecting animals from fish to amphibians, reptiles, birds, and mammals including humans, only one species is considered to be of widespread economic and public health importance. That species, Cryptosporidium parvum, is believed to be infectious for over 90 species of mammals including livestock and humans. It is also widely believed that cattle, especially preweaned calves, are a significant source of the fecal stage, the oocyst, that contaminates potable waters. Based on survey data a high percentage if not all herds are infected with Cryptosporidium parvum. Land application of manures is widely used to recycle nutrients for crop growth and animal feeding operations (AFOs) accumulate large quantities of manure. However, runoff from land applied manures and from lagoons or other fecal storage areas can result in contamination of surface and ground water. It is important to determine the conditions under which manure is a significant source of oocysts and to develop manure management strategies to prevent transport of Cryptosporidium parvum oocysts to source waters.

2. How serious is the problem? Why does it matter?

Cryptosporidium parvum, the causal agent of cryptosporidiosis, is a widespread protozoan parasite afflicting numerous mammalian species. Cryptosporidium parvum is also an important human pathogen as evidenced by dozens of waterborne outbreaks of cryptosporidiosis in the past

decade. The most severe outbreak was reported in Milwaukee, WI where over 400,000 people became ill and many died. Cryptosporidium parvum is a particularly serious health threat to immune compromised persons (e.g., AIDS, cancer patients) because there are no effective treatments for the disease. Cryptosporidiosis is also an economically important disease for livestock producers, especially dairy and beef cattle producers. Preweaned calves nationwide suffer scours, a prevalent diarrheal disease caused by one or several pathogens including Cryptosporidium, affecting growth, feed conversion, and mortality.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 201 Water Quality and Management (60%); National Program Area 302 Plant Biological and Molecular Processes (40%). Ensuring that waters are not contaminated by parasites or pathogens of agricultural origin that can cause human disease is an integral component of good water quality.

4. What was your most significant accomplishment this past year?

We have found that Cryptosporidium parvum oocysts adhere tenaciously to soil particles where oocysts rapidly decompose. These data indicate that once oocysts infiltrate into the soil profile the likelihood of leaching to groundwater or runoff to surface waters is remote. After surface water (river water) in close proximity to an animal feeding operation was examined and found contaminated with Cryptosporidium oocysts, beef cattle at that site were examined and nearly 30% were found to be excreting oocysts.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Established methods were tested and new methods were developed to optimize the detection and quantitation of oocysts in manure and soil. Development of these methods have enabled us to begin systematic studies on oocyst transport over ground surfaces and through soils of diverse types. Soil core studies indicated that vertical transport of oocysts through soils is limited because oocysts adhere to or become entrapped by soil particles. However, occlusion of soil pores by manure particles can interfere with water infiltration, enhancing the potential for runoff. Modification of field lysimeters (plots of ground with defined slope, soil type, and ground cover from which water can be collected) and construction of a rainfall simulator were completed. These will allow for systematic studies to assess the effects of slope, soil texture, and rainfall intensity/duration on rates/extent of surface transport of parasites/pathogens.
Examination of surface waters and oysters in those waters from 11 tributaries of the Chesapeake Bay were found contaminated with oocysts of Cryptosporidium parvum. One site in close proximity to an animal

feeding operation has repeatedly been examined and found heavily contaminated with Cryptosporidium oocysts. Nearly 30% of over 100 postweaned cattle (weighing 500 lbs or more) at that site were found to be excreting oocysts.

6. What do you expect to accomplish during the next year?

Studies will be conducted with field lysimeters and a rainfall simulator to investigate oocyst transport as a function of slope, soil texture, and rainfall intensity/duration. Watershed studies will be conducted

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assess actual transport under field conditions. Soil samples will be collected and analyzed from an animal feeding operation where adjacent surface water has been found contaminated with oocysts.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Information obtained from these studies have been shared with scientists from academia, the Centers for Disease Control, and the Environmental Protection Agency. As information becomes available on soil types, land slope, or management procedures that can have a significant impact on reducing environmental contamination with infectious agents, this will be shared with action agencies including extension agents who work with farmers.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Video, 20 minutes, 1500 copies distributed worldwide, produced by USDA Working Group on Water Quality, entitled "Cryptosporidiosis"

PUBLICATIONS:

01.
FAYER, R., TROUT, J. and JENKINS, M.C. 1998. Infectivity of Cryptosporidium parvum oocysts stored in water at environmental temperatures. Journal of Parasitology 84:1105-1108.

02.
GRACZYK, T.K., FAYER, R., CRANFIELD, M.R. and CONN, D.B. 1998. Recovery of waterborne Cryptosporidium parvum oocysts by freshwater benthic clams (Corbicula fluminea). Applied and Environmental Microbiology 64:427-430.

03.
FAYER, R., GRACZYK, T.K., LEWIS, E.J., TROUT, J.M. and FARLEY, C.A. 1998. Survival of infectious Cryptosporidium parvum in seawater and eastern ... Bay. Applied and Environmental Microbiology 64:1070-1074.

04.
GRACZYK, T.K., FARLEY, C.A., FAYER, R., LEWIS, E.J. and TROUT, J.M. 1998. Detection of Cryptosporidium oocysts and Giardia cysts in the tissues of ... diseases. Journal of Parasitology 84:1039-1042.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: IDENTIFICATION AND MAPPING OF GENES INVOLVED IN PARASITIC DISEASE RESISTANCE/SUSCEPTIBILITY

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Infectious diseases cost producers millions of dollars each year due to production losses and drug and vaccine costs. In addition, there is increasing field evidence of drug-resistant parasites. Thus, alternate control strategies are critically needed. This CRIS is aimed at using DNA marker-based methods to prevent these production losses by identifying animals which are genetically resistant to the infectious organisms. These studies should determine the specific chromosome that controls resistance and, eventually, the actual genes, and mechanisms through which animals become more resistant to specific infectious diseases.
Chickens: Avian coccidiosis is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Novel approaches for controlling this disease are urgently needed due to the lack of suitable current control strategies. Previous studies from our laboratory have shown that chickens with different genetic backgrounds display different levels of disease susceptibility and resistance to coccidiosis. This information led us to investigate the nature of chicken genes which control host immune responses. With recent advances in animal gene mapping techniques and the availability of chicken DNA markers which are useful in genotyping commercial broiler chickens, identification of gene markers associated with disease resistance to coccidiosis is now feasible. The long-term goal is to identify DNA markers associated with coccidiosis resistance in poultry. Once identified, these genetic markers will provide a novel tool for not only selecting coccidia-resistant chicken lines and but also eliminating potentially deleterious genes from the breeding stock. Genetic selection

strategies represent a logical, safe and environmentally friendly approach to disease control. If successful, this will enhance poultry productivity.

Swine: The initial thrust of the swine genome work was to help develop the basic swine genome map since at the beginning of this project only a rudimentary genome map was available for swine. We prepared new molecular genetic markers, termed microsatellite markers, for use as mapping reagents for pig genome studies. Since certain chromosomes are known to be associated with enhanced carcass traits our major effort was aimed at developing markers for these specific chromosomes. Thus we

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103 Animal Health 70%

developed techniques to isolate specific swine chromosome using laser based, sorting techniques and later chromosome microdissection procedures. As a result of these efforts, a panel of swine chromosome 6 markers were produced and a detailed map of this chromosome 6 developed. These markers have been incorporated into the overall swine genome maps and will be used to identify genes which are associated with improved pork meat characteristics and other carcass traits.

The major current thrust now focuses on determining whether swine which are more disease resistant can be identified. These studies are aimed at preventing parasite disease transmission through pork meat. Most consumers are aware that worms (Trichinella spiralis) contaminate pork products and thus they must pay attention to the cooking of this meat. Pork producers addressed this disease problem through their quality assurance program and improved management procedures. Now few, if any, pigs infected with Trichinella can be found. However, another foodborne parasite, Toxoplasma gondii, still is a problem for pork meat. Despite major efforts to prevent toxoplasmosis infections in pigs, cat fecal contamination of animal facilities and feed sources has resulted in continued low levels of this infection in U.S. pigs. Thus we have initiated studies to determine whether swine which are genetically resistant to Toxoplasma gondii infection can be identified. Conditions were developed so that deliberate infection studies with low doses of this parasite could be performed. Animals were tested for genetic resistance to this infection with the plan that the mechanism encoding this resistance could be determined. For analyses of mechanisms, a series of candidate genes have been targeted, specifically immune regulatory genes, the cytokine and the SLA genes. The role of specific genes, and the speed of their induction to stimulate immune responses, are being tested as controlling factors for genetic resistance. Wider analyses of swine genome markers to define all the genes which control resistance will be a later target of these studies.

Cattle: Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of the infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle

herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune

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system effectively reduces parasite transmission by reducing egg output by the female worms.
Our cattle studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. First, nothing is known concerning the exact mechanism that causes the resistance to infection. Secondly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. Third, we have recently shown that host genetics play an important role in determining if individual cattle become immune or not but, as of this date, little information concerning the genes involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of genetically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Chickens: Coccidiosis remains a major parasitic disease which costs poultry industry over $600 million annual economic losses. Although drugs and live parasites are being used to control coccidiosis in chickens, problems associated with incidence of drug-resistance and antigenic variations in the field strains of Eimeria parasites are increasing making these approaches less practical. Thus novel approaches for coccidiosis control are urgently needed for poultry industry.
Swine: Estimation of dollar losses to the industry due to swine parasitism is problematic, but $400 million per year is a mark that approximates production, management, and veterinary and pharmaceutical costs of parasite control. An additional concern is consumer confidence in pork products which is reduced by the threat of zoonosis from trichinellosis, toxoplasmosis, and secondary bacterial contamination of tissues. Toxoplasma gondii infection in swine creates a public health concern because this parasite can be transmitted to humans through the

handling and consumption of raw or undercooked meat. Current control methods rely heavily, if not exclusively, on management with variable results. Improved management practices for toxoplasmosis control at the farm level, such as the use of rodenticides instead of cats and intensive confinement of animals, probably explained the reduction in disease prevalence, from 24.2 % in the early 80's to 3.1 % in the 90's, in the last decade in market pigs. However, these management procedures have not been effective in totally preventing this infection or in substantially reducing the prevalence in older breeding animals. The latest regional surveys in Iowa and Illinois indicate prevalence in this

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population varies from 2.9% to 17% depending on the type of facility. Thus alternate methods of preventing this foodborne infectious disease are required. The genetic approach has the advantage that it aims to identify pigs which are naturally resistant to the disease. It also is not dependent on increased drug usage to control the disease.

Cattle: Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to$8 billion dollars per year. This cost in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss. At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of

efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a "normal" expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk. The only feasible and economically viable approach to heavy anthelmintic usage is the use of host genetics and immunity to control disease severity and transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without

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increased knowledge of the fundamental interaction of parasites and host genetics and immunity, novel control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 101 Animal Genome and Germplasm (70%); National Program Area 103 Animal Health (30%)
Genetic approaches will satisfy the need for the development of drug-free strategies to prevent infectious diseases. This should prove to be less expensive since this approach does not involve labor-intensive vaccination and would provide effective and sustainable methods to reduce parasite associated production losses. Identification of genes controlling protective anti-parasite immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance and potentially provide novel biotherapeutics for parasite control.

4. What was your most significant accomplishment this past year?

Chicken: Mapping studies of coccidiosis susceptible and resistant chickens have been initiated. We have initially evaluated 30 microsatellite markers which have been developed at the USDA East Lansing laboratory to identify genes associated with coccidiosis resistance traits in commercial broiler meat-type chickens. In our initial screening of microsatellite markers, 11 markers showed polymorphisms in the commercial chickens used for this cooperative study. Successful accomplishment of this project will lead to the development of gene-marker assisted control strategy for coccidiosis.
Swine: A microsatellite map for swine chromosome 6 was developed. Since chromosome 6 was known to be associated with enhanced carcass traits our efforts focused on developing more markers for this chromosome and, in the last year, to parts of this chromosome using very sensitive microdissection procedures. These markers have been incorporated into the international swine genome map.

Determined that swine which are genetically resistant to Toxoplasma gondii infection can be identified. Conditions were developed so that deliberate infection studies with low doses of T. gondii oocysts could be performed and samples collected from those animals can now being tested to determine the genes encoding this resistance.
Cattle: Completed typing genetically defined cattle for GI nematode susceptibility or resistance. Continued genomic DNA collection on cattle so that now DNA samples are available for three generations of selection of enhanced or diminished resistance to gastrointestinal nematode infection. Developed family structure for each animal to cover a

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minimum of seven generations prior to initiation of breeding program. Identified cattle genome markers to give adequate coverage of the genome for detailed mapping studies of these populations. These materials are being used to define genes encoding GI nematode resistance/susceptibility.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Early data focused on candidate genes, such as determining which animals show enhanced expression of specific cytokines following disease stress. This approach highlighted the potential use of new cloned genes as molecular biotherapeutics to decrease disease losses. Mapping data will now be developed to help producers select breeding stock which has better disease resistance and thus decrease morbidity and mortality caused by these infections. Overall, this research has expanded our understanding of the mechanisms through which animals resist infections and, thus, helped reduce our dependence on drugs and enhance our ability to select new methods to treat infections.
Chicken: We have identified and cloned candidate chicken genes encoding naturally-produced lymphocyte factors which mediate important immune functions, including recombinant chicken interferon-gamma which activates macrophages to enhance innate host immunity to coccidia parasites and Interleukin-15 (IL-15) which is an immune cell growth factor and promotes the growth of thymus-derived T-lymphocytes. T cells play a critical role in cell-mediated immune response to parasites. Studies on these recombinant lymphocyte-derived factors will enhance our knowledge on the basic immunology of host immune system-parasite interaction and lead to the development of novel genetic and immunological control strategies.
Swine: The initial thrust of the swine genome work was to help develop the basic swine genome map since at the beginning of this project only a rudimentary genome map was available for swine. We prepared new molecular markers, termed microsatellite markers for use as mapping reagents for pig genome studies. Since certain chromosomes are known to

be associated with enhanced carcass traits our major effort was aimed at developing markers for these specific chromosomes. Thus we developed techniques to isolate specific swine chromosome using laser based, sorting techniques. Later more detailed chromosome microdissection procedures were used to scrape out only small parts of individual chromosomes. As a result of these efforts, in collaboration with scientists at University of Minnesota and the Norwegian Veterinary College, a panel of swine chromosome 6 markers were produced and a detailed map of swine chromosome 6 developed. These markers have been incorporated into the international seine genome maps and shared with

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researchers at the Meat Animal Research Center (MARC), Clay Center, NE, and the international PiGMAP consortium. These microsatellite markers are now being tested to determine whether the genes on chromosome 6, which are associated with enhanced carcass traits, can be separated from the genetic defect that causes a potentially fatal stress syndrome in pigs. Additional studies determined the genetic complexity of the lines of swine leukocyte antigen (SLA) defined miniature pigs used for our parasite resistance studies. Using a broad panel of swine genome markers DNA from these lines were assessed for their genetic complexity. Determined that swine which are genetically resistant toparasite (Toxoplasma gondii) infection can be identified. Our earlier studies had proven that Trichinella spiralis resistant pigs could be identified. Since trichinosis can now be almost completely controlled by proper management, our studies on swine resistance to T. spiralis infection were discontinued and analyses of T. gondii initiated in 1995. Conditions were developed so that deliberate infection studies with low doses of T. gondii oocysts could be performed. Samples were collected from those animals to determine whether 1)genetic resistance could be found and 2)the mechanism encoding this resistance determined. Preliminary evidence for T. gondii resistant pigs has been found. Now studies are underway to test a series of candidate genes have been targeted, specifically the immune regulatory, cytokine genes and the SLA genes. Early studies indicated that SLA genes may be one factor in determining the degree of parasite resistance. This was not confirmed in later studies. Detailed analyses of the speed of the immune response, particularly of the cytokine interferon-gamma (IFNg) expression, indicates that this will be a controlling factor. Measurement of other cytokine gene expression during T. gondii infection is in progress. Cattle: After significant exposure to the GI nematodes most cattle in a herd demonstrate immunity against the parasites. This immunity can be manifested as resistance to reinfection or as reductions in the egg output from resident worms. Both responses are important in limiting the spread of the infection. Although most cattle demonstrate effective immune responses, a small percentage do not demonstrate a significant immune response. This percentage is estimated to be between 15 and

25%.
Demonstrated that the ability to mount such immunity to gastrointestinal nematodes is strongly influenced by host genetic factors. The odds of producing calves of the susceptible phenotype for certain sires is approximately 20 times that of other sires. Using this information, selective breedings were done to produce cattle of the susceptible phenotype as well as cattle that are strongly resistant to infection. These breedings used Black Angus cattle that had already been selected for identity at their major histocompatibility complex (BoLA). Work done as part of this project indicated that BoLA itself has little

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effect of the immunity being measured. Calves produced as a result of the selective breeding were then tested for their ability to mount immunity to the parasites by exposure of the weaned calves to infected pastures for 120 days. During this test period calves were extensively monitored for parasite burdens, and for anti-parasite immunity. At the end of the test period some calves were killed for more extensive work-up both parasitologically and immunologically, and others were used to continue the breeding program. Such measures resulted in over 2000 data points per animal. This procedure has continued to a point that there are now 3 generations of animals upon which there is extensive phenotypic data. At the same time DNA has been collected and saved from each individual.

Total numbers of animals for which such data is available is now just over 180, and there are currently 41 additional animals on test. In collaborative studies with other ARS laboratories, a series of probes have been identified that bind to polymorphic sites in the genome of the study population, and that adequately cover the entire bovine genome.

Other collaborations have developed software and analysis tools to define family structure for each test animal that covers at least 7 generations.

Genotyping with the identified probes has begun. The genotypic data will be combined with phenotypic data and family structure data for analyses aimed at mapping genetic regions that control immunity to the parasites.

6. What do you expect to accomplish during the next year?

Chicken: Mapping studies of coccidiosis susceptible and resistant chickens have identified markers which show polymorphisms in the commercial chickens used for this cooperative study. These studies will be extended in the next year to look at many more markers and to begin to define the chromosomes and the exact genes which control coccidiosis resistance and susceptibility. Successful accomplishment of this project will lead to the development of gene-marker assisted control strategy

for coccidiosis. Advances in technology in molecular gene mapping and chicken gene cloning will enable comprehensive understanding of genetic control of disease resistance to coccidiosis and lead to a logical genetic control strategy against poultry diseases.

Swine: Determination of the genes which control T. gondii resistance will require testing many more pigs to define the exact genetic locations controlling resistance. As candidate genes involved in resistance are identified, studies are planned to determine their applicability in preventing toxoplasmosis in both inbred and outbred populations of pigs.

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Currently most pigs which are resistant have fewer parasites in their tissues. For foodborne protozoan parasite infections it remains to be determined whether genetic resistance must be complete, i.e., whether no parasite can be left in the tissue or whether significant decreases in parasite burden is effective. Thus we will perform studies to test an alternate combined approach to controlling this infection, involving combination of genetic control with vaccination.
Because the swine industry has been so effective in controlling parasitic infections in their modern facilities our group has expanded our research into detailed analyses of the developing immune system of the neonatal pig. In collaboration with PIC USA studies are underway to assess mucosal immunity in neonatal pigs. These data are being collected on pigs from defined genetic lines so that future studies can be performed to correlate parameters associated with overall disease resistance with the genetic alleles that regulate mucosal immunity and the related cytokine responses.
Cattle: Targeted gene expression studies will continue to identify the candidate genes which are expressed by cattle which are genetically resistant to GI nematode infections. By comparing gene expression in infected susceptible and resistant animals we will be able to identify altered activity of the genes which interact to control parasite responses. This will lead to identification of novel control mechanisms, and of the complex interplay between genes, involved in disease responses. Efforts will also be started to begin comparative mapping of susceptible and resistant animals to identify novel genes which control cattle anti-parasite responses.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

All our genome efforts have been coordinated with national genome mapping efforts. Publications have included references to the publically available databases that can be accessed through the Internet. Any

scientist at universities or commercial facilities can readily apply our gene cloning and mapping data to their own efforts.
For each species we have set up collaborations with university and, where appropriate, commercial partners to develop the technology as quickly as possible. Due to a large numbers of genetic (microsatellite) markers that need to be tested and the labor-intensive nature of molecular mapping technology, there is a time constraints to completing testing for markers associated with disease resistance. It will take many years to confirm gene associations to fully transfer this technology to the poultry and livestock producers.

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For our cloned and expressed candidate gene products we have established commercial agreements and new grants to make these as widely available to users.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Genetic Resistance to parasites. BEEF Magazine. page 56. September 1998.
HARTSOCK, T. 1998. Pig Tales. Take a look in your backyard. The Delmarva Farmer. Section 3, p3.
Making Coccidia Less Cocky. Agricultural Research, January 1999. pp.20-21.

PUBLICATIONS:

01.
SONG, K.D., LILLEHOJ, H.S., CHOI, K.D., ZALENGA, D. and HAN, J.Y. 1997. Expression and functional characterization of recombinant chicken interferon-gamma. Vet. Immunol. Immunopathol. 58:321-333.

02.
ALLEN, P. and LILLEHOJ, H.S. 1998. Genetic influence on nitric oxide production during Eimeria tenella infections in chickens. Avian Disease 42:397-403.

03.
SASAI, K., YOSHIMURA, K., LILLEHOJ, H., WITHANAGE, G.S.K., FUKATA, T., BABA, E. and ARAKAWA, A. 1997. Analysis of splenic and thymic lymphocyte ... enteritidis. Vet. Immunol. Immunopathol. 59:359-367.

04.
LILLEHOJ, H.S. and CHOI, K.D. 1998. Molecular and functional characterization of a novel chicken cytokine: its ... resistance.
Proc of 135th Annual Convention of American Vet Med. Assn. 135:186.

05.

LUNNEY, J.K. and BUTLER, J.E. 1998. Immunogenetics, pp.163-197. IN: M.F. Rothschild and A. Ruvinsky (eds) Genetics of the Pig. CAB Intnl., Wallingford, UK.

06.
LUNNEY, J.K. 1998. Cytokines orchestrating the immune response. OIE Scientific and Technical Review. 17:84-94.

07.
PESCOVITZ, M.D., LUNNEY, J.K., BOYD, P., WALKER, J., LEE, R. and SAALMUELLER, A. 1998. Analyses of monoclonal antibodies reacting with porcine CD5: Workshop. Vet. Immunol. Immunopathol. 60:269-274.

Publications: (Continued)

08.
PESCOVITZ, M.D., BOOK, B.K., LUNNEY, J.K., BOYD, P., WALKER, J., LEE, R., PETRINEC, N. and SAALMUELLER, A. 1998. Analyses of ... wCD6: Results 2nd. Int'l Swine CD Workshop. Vet. Immunol. Immunopathol. 60:285-290.

09.
SAALMUELLER, A., PAULY, T., HAVERSON, K., BOYD, P. and LUNNEY, J.K. 1998. Summary of the first round analyses of the Second International Swine CD Workshop. Vet. Immunol. Immunopathol. 60:237-250.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400116 Year: 98 Project Number: 1265-31320-012-02 R
Mode Code: 1265-20-00 STP Codes: 3.1.2.1 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: DEVELOPMENT OF REAGENTS FOR IDENTIFICATION OF PIG CHROMOSOME 6 ASSOCIATED QUANTITATIVE TRAIT LOCI

Period Covered From: 01/98 To: 08/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

This cooperative agreement with University of Minnesota was aimed at using DNA marker-based methods to map genes associated with improved carcass and meat traits in pigs. The initial thrust of this swine genome work was to help develop the basic swine genome map since at the beginning of this project only a rudimentary genome map was available for swine. We prepared new molecular genetic markers, termed microsatellite markers, for use as mapping reagents for pig genome studies. Since certain chromosomes are known to be associated with enhanced carcass traits our major effort was aimed at developing markers for these specific chromosomes. Thus we developed techniques to isolate specific swine chromosome using laser based, sorting techniques. Later, even smaller pieces of chromosomes were used to develop markers at specific locations using what are termed chromosome microdissection procedures. As a result of these efforts, a panel of swine chromosome 6 markers were produced and a detailed map of this chromosome 6 developed. These markers have been incorporated into the overall swine genome maps developed internationally. These markers can now be used to identify genes which are associated with improved pork meat characteristics and other carcass traits.

2. How serious is the problem? Why does it matter?

Livestock genetic improvement studies have traditionally involved years of planned breedings and recording of production and meat quality traits. This is very expensive and time consuming. In the last 10 years the national animal genome projects have been developed. With new

molecular genome linkage maps scientists are now able to identify and localize genes that regulate important production traits known as quantitative or economic, trait loci (QTL or ETL). Certain swine chromosomes were known to be associated with enhanced carcass traits, our efforts were first made to target these chromosomes and produce panels of markers to improve the likelihood of matching a superior trait with a known chromosome marker. These studies were then aimed at using a broader panel of swine genome markers to define the genetic complexity of inheritance for improved meat quality. Availability of these markers now means that specific genetic tests can be performed to define

Accession: 0400116 Year: 98 Project Number: 1265-31320-012-02 R
Mode Code: 1265-20-00 STP Codes: 3.1.2.1 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

linkages of these markers to carcass traits. In the future this will enable pork producers to choose pigs that bear the most desirable genes.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 101 Animal Genome and Germplasm (100%) Genetic approaches will provide markers that identify inherited traits of pigs. These can be used to find the exact genetic location of a gene and to determine which pigs express the best type of that gene. This information can then be used to help breeders to identify their superior genetic stock. Identification of genes controlling carcass traits, and the mechanisms by which they function, will allow the identification and manipulation of genes controlling meat quality.

4. What was your most significant accomplishment this past year?

A microsatellite map for swine chromosome 6 was developed. Since chromosome 6 was known to be associated with enhanced carcass traits our efforts focused on developing more markers for this chromosome and, in the last year, to parts of this chromosome using very sensitive microdissection procedures. These markers have been developed and incorporated into the international swine genome map.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The initial thrust of the swine genome work was to help develop the basic swine genome map since at the beginning of this project only a rudimentary genome map was available for swine. We prepared new molecular markers, termed microsatellite markers, for use as mapping reagents for pig genome studies. Since certain chromosomes are known to be associated with enhanced carcass traits our major effort was aimed at developing markers for these specific chromosomes. Thus we developed techniques to isolate specific swine chromosomes using laser based,

sorting techniques. Later more detailed chromosome microdissection procedures were developed to scrape out only small parts of individual chromosomes. As a result of these efforts, in collaboration with scientists at University of Minnesota and the Norwegian Veterinary College, a panel of swine chromosome 6 markers were produced and a detailed map of swine chromosome 6 developed. These markers have been incorporated into the international swine genome maps and shared with researchers at the Meat Animal Research Center (MARC), Clay Center, NE, and the international PiGMAP consortium. These microsatellite markers are now being tested to determine whether the genes on chromosome 6,

Accession: 0400116 Year: 98 Project Number: 1265-31320-012-02 R
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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

which are associated with enhanced carcass traits, can be separated from the genetic defect that causes a potentially fatal stress syndrome in pigs. Additional studies determined the genetic complexity of the lines of swine leukocyte antigen (SLA) defined miniature pigs used for our parasite resistance studies. Using a broad panel of swine genome markers DNA from these lines were assessed for their genetic complexity.

6. What do you expect to accomplish during the next year?

This grant is now finished.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

All our genome efforts have been coordinated with national genome mapping efforts. Publications have included references to the publically available databases that can be accessed through the Internet. Any scientist at universities or commercial facilities can readily apply our gene cloning and mapping data to their own efforts.
For each species we have set up collaborations with university and, where appropriate, commercial partners to develop the technology as quickly as possible. Due to a large numbers of genetic (microsatellite) markers that need to be tested and the labor-intensive nature of molecular mapping technology, there is a time constraints to completing testing for markers associated with carcass traits. It will take many years to confirm gene associations to fully transfer this technology to the pork producers.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
RAMSOONDAR, J.J., RUCKER, E.B., VASQUEZ, J.C., GALLAGHER, D.S., GRIMM, D.R., LUNNEY, J.K., SCHOOK, L.B. and PIEDRAHITA, J.A. 1998. Isolation and genetic characterization of the ... gene. Anim. Genetics. 29:43-47.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401442 Year: 98 Project Number: 1265-31320-012-03 R
Mode Code: 1265-20-00 STP Codes: 3.1.2.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: DNA MARKER TECHNOLOGY IN COMMERCIAL BROILER BREEDER SELECTION PROGRAMS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Avian coccidiosis is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Due to the lack of suitable control strategies for coccidiosis, novel approaches are urgently needed. Previous studies from our laboratory have shown that host genes control the level of disease susceptibility and resistance to coccidiosis. This information led us to investigate the identity of chicken genes which control host immune responses to coccidia parasites.
With recent advances in animal gene mapping techniques and the availability of chicken DNA markers which are useful in genetically typing commercial broiler chickens, identification of gene markers associated with disease resistance to coccidiosis is now feasible. The overall long-term goal of this project which is being funded by the Fund For Rural America is to identify DNA markers associated with coccidiosis and Marek's Disease resistance in poultry. Once identified, these genetic markers will provide a novel tool for not only selecting coccidia-resistant chicken lines and but also eliminating potentially deleterious genes from the breeding stock. Genetic selection strategies represent a logical, safe and environmentally friendly approach and if successful, will enhance poultry productivity.

2. How serious is the problem? Why does it matter?

Coccidiosis remains a major parasitic disease which costs the poultry industry over $600 million annually economic losses. Although drugs and live parasites are being used to control coccidiosis in chickens,

incidence of drug-resistant coccidia strains is increasing. Furthermore, there is an increasing incidence of field parasite strains which are antigenically different from, and much more virulent than, the live vaccine strains. These problems make the current prevention approaches for coccidiosis unsuitable for long-term usage. Thus novel approaches for coccidiosis control are urgently needed for poultry industry.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401442 Year: 98 Project Number: 1265-31320-012-03 R
Mode Code: 1265-20-00 STP Codes: 3.1.2.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 103 Animal Health & Genome (70%); National Program Area 101 Animal Genome (30%)
Genetic approaches to disease control will satisfy the need for the development of drug-free control strategies for avian coccidiosis and should prove to be less expensive since the genetic selection of coccidiosis-resistant chickens does not involve labor-intensive vaccination.

4. What was your most significant accomplishment this past year?

During the last year, we have evaluated 30 different gene markers which have been developed at the USDA East Lansing laboratoryon commercial broilers. These studies compared chicken lines with the plan to identify genes associated with coccidiosis resistance traits in commercial broiler meat-type chickens. In our initial screening of 200 genetic markers, 11 genes turned out to be useful in differentiating commercial chicken lines.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Scientific advance in poultry genome mapping has been slow compared to that in other animal species. During the last several years, however, sufficient numbers of genetic markers were developed and it is now feasible to conduct a meaningful chicken genome mapping studies. As the more advanced technology in molecular gene mapping becomes available, we will be able to test more genetic markers at a faster rate to develop efficient mapping strategy for coccidiosis disease resistance. Successful accomplishment of this project will lead to the development of gene-marker assisted control strategy for coccidiosis.

6. What do you expect to accomplish during the next year?

Identification of potential genetic markers which are associated with coccidiosis disease resistance and susceptibility will be conducted by

screening newly developed genetic markers. We are collaborating with a commercial poultry geneticists from Purdue Farms and with scientists at the University of Delaware. Due to a large numbers of genetic markers that need to be tested and the labor-intensive nature of molecular mapping technology, there is a time constraint to test many markers associated with coccidiosis disease resistance.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401442 Year: 98 Project Number: 1265-31320-012-03 R
Mode Code: 1265-20-00 STP Codes: 3.1.2.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Due to our close working relationship with a local broiler industry, any meaningful results will be quickly transferred to Purdue Farms Inc.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Genetic Basis for Resistance and Immunity to Avian Diseases, Northeastern 60 technical Committee Report, pp1-11, 1998.

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400112 Year: 98 Project Number: 1265-32000-044-06 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: STRATEGIC CONTROL OF GASTROINTESTINAL NEMATODES UNDER INTENSIVE ROTATIONAL GRAZING

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms.
These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that causes the resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. We have recently also shown that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes

involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to $8 billion dollars per year. This

Accession: 0400112 Year: 98 Project Number: 1265-32000-044-06 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

cost in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss.

At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a normal expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk.

The only feasible and economically viable approach to heavy anthelmintic usage is the use of the host immune system to control disease severity

and transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without increased knowledge of the fundamental interaction of parasites and host immune system components, such immunity-based control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400112 Year: 98 Project Number: 1265-32000-044-06 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 101 Animal Genomes, Germplasm, and Development (30%); National Program Area 103 Animal Health (70%).
These studies add basic and applied information concerning effective and sustainable methods to reduce parasite associated production losses. The use of host immunity in an integrated control program will improve the efficacy of cattle production systems, and result in overall healthier animals by reducing stress associated with parasitic infections, and by removing immunomodulatory pressure exerted by the parasites. In addition, identification of protective immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance.

4. What was your most significant accomplishment this past year?

Demonstrated that in a working dairy practicing intensive rotational grazing parasite treatments aimed at reducing pasture contamination were much more effective in reducing parasite-induced production losses than were programs aimed at treatment of the animals. In the past year a strategic treatment program was applied for a second consecutive year to verify results seen in the first year of such a program. This was necessary because a single year's data is suspect because of potential environmental and nutritional differences seen in such production systems. The results of a second year were even more dramatic than the first year probably because of a carry-over effect from the first year on the number of parasites on the pastures. Comparing 2 years of strategic treatments with 2 years of therapeutic treatments indicates an overall increase of 2-3 pounds of milk/animal/day over the time frame of the experiment.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Five years ago we were approached by a dairy farmer from south-central PA. This farmer was an early adherent to intensive rotational grazing for dairy cows. He had been practicing such a system for 6 years, but

had begun to notice that his milk production was dropping in the summer, and he was concerned that the cause was internal parasites. We began to monitor his herd for GI nematodes. Two years of monitoring showed that parasites were the problem, and that the major parasite was Ostertagia. We then developed a treatment regimen the goal of which was to reduce the number of parasites on the pastures. We demonstrated that the intensive rotational grazing systems in use was very beneficial to the parasites. The reason for this is the fact that optimal conditions for parasite growth and survival are similar to optimal conditions for grass growth. As such intensive grazing systems that maximize pasture

Accession: 0400112 Year: 98 Project Number: 1265-32000-044-06 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

utilization may also greatly enhance parasite transmission. Adoption of this program resulted in an average increase of 2 pounds of milk/animal/day over the entire calendar year. These studies have provided the basis for future testing of control programs based on utilization of the host immune system.
In the course of performing this very applied research protocol we have also demonstrated a number of basic concepts in the biology of the parasites. We have found that immunity to GI nematodes can be manifested in a number of ways. The most common response limits the number of parasites that become established in the host. In most nematode species this response is evident after an exposure of the cattle to infected pastures for 3-6 months. In the case of Ostertagia, this response does not appear for many months, and instead previously infected cattle respond with immune responses that limit the size of the worms, and the number of eggs laid by the female worms. Because immunity can be manifested in a number of ways we have found the commonly used method of assessing parasite infections, the enumeration of all species of parasite eggs in the feces, greatly underestimates Ostertagia numbers in cattle. They have also confirmed a genetic control of immune responses, and the idea that the parasites are "over dispersed" in cattle herds. These studies have also resulted in a more careful description of seasonal transmission patterns for the important cattle nematodes, and have demonstrated that the ability of Ostertagia to overwinter on US pastures has been greatly underestimated.

6. What do you expect to accomplish during the next year?

Goals for the ensuing year are to continue to define the interaction of the host immune system and GI nematodes under field conditions. These studies will include continued monitoring of production animals for parasite burdens, and for production parameters. In the case of dairy animals this will entail analyses of milk production records. The effect of various treatment and management options will be assessed not only on production, and parasite loads, but also on parasite transmission and the appearance of immune responses against the

parasites in the young animals. We will use our previous data that defines seasonal transmission patterns at the given locations to assess changes.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

In the past Year: The results of these studies have been presented to a

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103 Animal Health 70%

number of groups involving, the American Veterinary Medical Association, the Beef Improvement Federation, the International four-H Youth Exchange, Grassland Specialists of USDA, REE, CSREES, the Grasslands Conservation Initiative, and a Graduate Seminar of the Virginia-Maryland College of Veterinary Medicine. In addition these results have been presented as a radio interview with station KFIZ in Fond du Lac, Wisconsin. Discussed results of studies with writer for the Progressive Farmer for a future article on the work.
The scientist involved has answered numerous phone calls from cattle producers who have heard about the work, and has discussed in length these results with cattle individual cattle producers in Maryland, Missouri, New Hampshire, Pennsylvania, West Virginia, Virginia, and Kentucky. Presented training session to practicing Veterinarians from the Southwestern U.S. on practical parasite control in San Diego, CA in February 1998. The training session was supported by Pfizer, Animal Health as part of their program of technology transfer to field situations.
Larvae of parasitic nematodes were supplied to scientists at Pennsylvania State University, Montana State University, and USDA, ARS, PS&WMRL, University Park, PA. These larvae were used in research programs at these institutions.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

An Ounce of Prevention Equals a pound of milk. Agricultural Research. pages 10-11. January 1998.
Count Your Eggs Before They Hatch. BEEF Magazine. Pages 8-12. April 1998.
Genetic Resistance to parasites. BEEF Magazine. page 56. September 1998.

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99

Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149917 Year: 98 Project Number: 1265-32000-044-07 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: EXPRESSION OF BOVINE IL-12 AND IL-15, AND THEIR ROLE IN INFECTIOUS DISEASE

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms.
These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that causes the resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. We have recently also shown that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes

involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to$8 billion dollars per year. This cost

Accession: 0149917 Year: 98 Project Number: 1265-32000-044-07 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss.

At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a normal expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk. The only feasible and economically viable approach to heavy anthelmintic usage is the use of the host immune system to control disease severity and

transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without increased knowledge of the fundamental interaction of parasites and host immune system components, such immunity-based control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Accession: 0149917 Year: 98 Project Number: 1265-32000-044-07 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 101 Animal Genomes, Germplasm, and Development (30%); National Program Area 103 Animal Health (70%).
These studies add basic and applied information concerning effective and sustainable methods to reduce parasite associated production losses. The use of host immunity in an integrated control program will improve the efficacy of cattle production systems, and result in overall healthier animals by reducing stress associated with parasitic infections, and by removing immunomodulatory pressure exerted by the parasites. In addition, identification of protective immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance.

4. What was your most significant accomplishment this past year?

Immune regulators, termed cytokines or interleukins, have been cloned. We assessed the effect of administration of recombinant bovine Interleukin-12 (IL12) on the neonatal bovine gut, and on the course of a primary Cryptosporidium parvum infection in neonatal calves. Because Cryptosporidium parvum is such a serious problem in newborn calves, and current thinking dictates that immunity to this parasite is dependent of T lymphocyte responses, we used this parasite to determine if IL12 could be used as a functional immunomodulator in cattle. Found that administration of IL12 subcutaneously resulted in increased numbers of mature T lymphocytes in lymph nodes draining the gut, but not in the mucosal tissues of the gut. Because the effect was not seen at the mucosa, the administration of IL12 had no significant effect upon a primary Cryptosporidium parvum infection.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Over the life of this project we have cloned and expressed in vitro the gene for bovine Interleukin 12. We demonstrated that the gut immune system of newborn calves is almost devoid of mature T lymphocytes, and that administering IL12 to newborn calves can increase the number of T

lymphocytes in lymph nodes associated with the gut. Our results indicated that the treatment of C. parvum infected animals with IL12 did exert significant biological effects in calves, but that procedures that target activity more specifically to the gut surface are necessary before this approach can be utilized for this parasite species.

6. What do you expect to accomplish during the next year?

Work will be expanded to determine if the administration of recombinant IL12 can effect a primary Ostertagia infection. The protective immune

Accession: 0149917 Year: 98 Project Number: 1265-32000-044-07 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

response to GI nematodes usually involves an immediate hypersensitivity response. We have shown that such a response is ineffective for Ostertagia. We will try to stimulate a strong delayed-type hypersensitivity response in the abomasal mucosa of Ostertagia-infected calves, and assess the effect on the parasite. We will also explore new methods to target the activity of recombinant cytokines such as IL12 to the gut surface. This effort will focus on the construction of bacterial vectors that can colonize the gut while expressing the bovine cytokine genes.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

In the past Year: Reagents generated to study immune responses in bovine have been made available to the research community and remain as such. To date, over 100 requests for reagents from scientists throughout the world have been filled. The cloning of a functional IL12 cytokine has lead to corporate interest and involvement in synthesizing this reagent and making it available for research and commercial use following a thorough and ongoing investigation at the USDA of its application as a general immune stimulator to attenuate certain classes of infectious agents of cattle. Constraints to the application of these technologies i.e. genetically synthesized cytokines, are related to the present lack of information on the effectiveness of their use as general immune stimulators for attenuating parasitic infections (cytokines).

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400841 Year: 98 Project Number: 1265-32000-044-09 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: STRATEGIC CONTROL OF NEMATODES IN DAIRY CATTLE ON INTENSIVE ROTATIONAL GRAZING

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms.
These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that causes the resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. We have recently also shown that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes

involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to $8 billion dollars per year. This

Accession: 0400841 Year: 98 Project Number: 1265-32000-044-09 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

cost in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss.

At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a normal expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk.

The only feasible and economically viable approach to heavy anthelmintic usage is the use of the host immune system to control disease severity

and transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without increased knowledge of the fundamental interaction of parasites and host immune system components, such immunity-based control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400841 Year: 98 Project Number: 1265-32000-044-09 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 101 Animal Genomes, Germplasm, and Development (30%); National Program Area 103 Animal Health (70%).
These studies add basic and applied information concerning effective and sustainable methods to reduce parasite associated production losses. The use of host immunity in an integrated control program will improve the efficacy of cattle production systems, and result in overall healthier animals by reducing stress associated with parasitic infections, and by removing immunomodulatory pressure exerted by the parasites. In addition, identification of protective immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance.

4. What was your most significant accomplishment this past year?

Showed that in field conditions immunity to most GI nematodes arises fairly rapidly. Exposure to infected pastures for 3-6 months induces immune responses that significantly reduce the number of parasites that become established after subsequent infection. The exception to this effective immunity is Ostertagia ostertagi. After a similar exposure period there is a stunting of the worms, and a reduced egg output by the females, but worm numbers are unaffected, and as a result cattle remain at risk for parasite-induced production losses caused by this parasite for an extremely prolonged time.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

In the course of determining the basis for immunity to Ostertagia we have found that immunity to GI nematodes can be manifested in a number of ways. The most common response limits the number of parasites that become established in the host. In most nematode species this response is evident after an exposure of the cattle to infected pastures for 3-6 months. In the case of Ostertagia, this response does not appear for many months, and instead previously infected cattle respond with immune responses that limit the size of the worms, and the number of eggs laid

by the female worms. Because immunity can be manifested in a number of ways we have found the commonly used method of assessing parasite infections, the enumeration of all species of parasite eggs in the feces, greatly underestimates Ostertagia numbers in cattle. Co-operative studies with University and industry scientists and producers have begun to take the principles discerned in the laboratory to field situations. These studies have confirmed the presence of different kinds of immune responses to the different parasites, and have begun to define the level of exposure necessary to elicit the responses. They have also confirmed a genetic control of immune responses, and the

Accession: 0400841 Year: 98 Project Number: 1265-32000-044-09 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

idea that the parasites are over dispersed in cattle herds. These studies have also resulted in a more careful description of seasonal transmission patterns for the important cattle nematodes, and have demonstrated that the ability of Ostertagia to overwinter on US pastures has been greatly underestimated. Additionally we have demonstrated that intensive rotational grazing systems can be very beneficial to the parasites. The reason for this is the fact that optimal conditions for parasite growth and survival are similar to optimal conditions for grass growth. As such intensive grazing systems that maximize pasture utilization may also greatly enhance parasite transmission. We have demonstrated that it is imperative that producers evolve parasite control programs that minimize the build-up of parasites on pasture, rather than programs that treat for parasite-induced losses. Studies in a working dairy have shown that such treatments can result in an average increase of 2 pounds of milk/animal/day over the entire calendar year. These studies have provided the basis for future testing of control programs based on utilization of the host immune system.

6. What do you expect to accomplish during the next year?

This project is now terminated.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

In the past Year: The results of these studies have been presented to a number of groups involving, the American Veterinary Medical Association, the Beef Improvement Federation, the International four-H Youth Exchange, Grassland Specialists of USDA, REE, CSREES, the Grasslands Conservation Initiative, and a Graduate Seminar of the Virginia-Maryland College of Veterinary Medicine. In addition these results have been presented as a radio interview with station KFIZ in Fond du Lac, Wisconsin. Discussed results of studies with writer for the Progressive

Farmer. This discussion will be the basis for a future article on the work.

The scientist involved has answered numerous phone calls from cattle producers who have heard about the work, and has discussed in length these results with cattle individual cattle producers in Maryland, Missouri, New Hampshire, Pennsylvania, West Virginia, Virginia, and Kentucky. Presented training session to practicing Veterinarians from the Southwestern U.S. on practical parasite control in San Diego, CA in February 1998. The training session was supported by Pfizer, Animal Health as part of their program of technology transfer to field

Accession: 0400841 Year: 98 Project Number: 1265-32000-044-09 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

situations.
Larvae of parasitic nematodes were supplied to scientists at Pennsylvania State University, Montana State University, and USDA, ARS, PS&WMRL, University Park, PA. These larvae were used in research programs at these institutions.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

An Ounce of Prevention Equals a pound of milk. Agricultural Research. pages 10-11. January 1998.
Count Your Eggs Before They Hatch. BEEF Magazine. Pages 8-12. April 1998.
Genetic Resistance to parasites. BEEF Magazine. page 56. September 1998.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400844 Year: 98 Project Number: 1265-32000-044-10 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: EFFECT OF INTENSIVE ROTATIONAL GRAZING ON NEMATODE INFECTIONS IN DAIRY CATTLE

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms.
These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that causes the resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. We have recently also shown that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes

involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to $8 billion dollars per year. This

Accession: 0400844 Year: 98 Project Number: 1265-32000-044-10 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

cost in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss.

At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a normal expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk.

The only feasible and economically viable approach to heavy anthelmintic usage is the use of the host immune system to control disease severity

and transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without increased knowledge of the fundamental interaction of parasites and host immune system components, such immunity-based control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400844 Year: 98 Project Number: 1265-32000-044-10 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 101 Animal Genomes, Germplasm, and Development (30%); National Program Area 103 Animal Health (70%).
These studies add basic and applied information concerning effective and sustainable methods to reduce parasite associated production losses. The use of host immunity in an integrated control program will improve the efficacy of cattle production systems, and result in overall healthier animals by reducing stress associated with parasitic infections, and by removing immunomodulatory pressure exerted by the parasites. In addition, identification of protective immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance.

4. What was your most significant accomplishment this past year?

Demonstrated that in a working dairy practicing intensive rotational grazing parasite treatments aimed at reducing pasture contamination were much more effective in reducing parasite-induced production losses than were programs aimed at treatment of the animals. In the past year a strategic treatment program was applied for a second consecutive year to verify results seen in the first year of such a program. This was necessary because a single year s data is suspect because of potential environmental and nutritional differences seen in such production systems. The results of a second year were even more dramatic than the first year probably because of a carry-over effect from the first year on the number of parasites on the pastures. Comparing 2 years of strategic treatments with 2 years of therapeutic treatments indicates an overall increase of 2-3 pounds of milk/animal/day over the time frame of the experiment.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

In the course of determining the basis for immunity to Ostertagia we have found that immunity to GI nematodes can be manifested in a number of ways. The most common response limits the number of parasites that

become established in the host. In most nematode species this response is evident after an exposure of the cattle to infected pastures for 3-6 months. In the case of Ostertagia, this response does not appear for many months, and instead previously infected cattle respond with immune responses that limit the size of the worms, and the number of eggs laid by the female worms. Because immunity can be manifested in a number of ways we have found the commonly used method of assessing parasite infections, the enumeration of all species of parasite eggs in the feces, greatly underestimates Ostertagia numbers in cattle. Cooperative studies with University and industry scientists and producers have begun

Accession: 0400844 Year: 98 Project Number: 1265-32000-044-10 S
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

to take the principles discerned in the laboratory to field situations. These studies have confirmed the presence of different kinds of immune responses to the different parasites, and have begun to define the level of exposure necessary to elicit the responses. They have also confirmed a genetic control of immune responses, and the idea that the parasites are over dispersed in cattle herds. These studies have also resulted in a more careful description of seasonal transmission patterns for the important cattle nematodes, and have demonstrated that the ability of Ostertagia to overwinter on US pastures has been greatly underestimated. Additionally we have demonstrated that intensive rotational grazing systems can be very beneficial to the parasites. The reason for this is the fact that optimal conditions for parasite growth and survival are similar to optimal conditions for grass growth. As such intensive grazing systems that maximize pasture utilization may also greatly enhance parasite transmission. We have demonstrated that it is imperative that producers evolve parasite control programs that minimize the build-up of parasites on pasture, rather than programs that treat for parasite-induced losses. Studies in a working dairy have shown that such treatments can result in an average increase of 2 pounds of milk/animal/day over the entire calendar year. These studies have provided the basis for future testing of control programs based on utilization of the host immune system.

6. What do you expect to accomplish during the next year?

This project is terminated.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

In the past Year: The results of these studies have been presented to a number of groups involving, the American Veterinary Medical Association, the Beef Improvement Federation, the International four-H Youth

Exchange, Grassland Specialists of USDA, REE, CSREES, the Grasslands Conservation Initiative, and a Graduate Seminar of the Virginia-Maryland College of Veterinary Medicine. In addition these results have been presented as a radio interview with station KFIZ in Fond du Lac, Wisconsin. Discussed results of studies with writer for the Progressive Farmer. This discussion will be the basis for a future article on the work.

The scientist involved has answered numerous phone calls from cattle producers who have heard about the work, and has discussed in length these results with cattle individual cattle producers in Maryland,

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

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Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Missouri, New Hampshire, Pennsylvania, West Virginia, Virginia, and Kentucky. Presented training session to practicing veterinarians from the Southwestern U.S. on practical parasite control in San Diego, CA in February 1998. The training session was supported by Pfizer, Animal Health as part of their program of technology transfer to field situations.
Larvae of parasitic nematodes were supplied to scientists at Pennsylvania State University, Montana State University, and USDA, ARS, PS&WMRL, University Park, PA. These larvae were used in research programs at these institutions.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

An Ounce of Prevention Equals a pound of milk.. Agricultural Research. pages 10-11. January 1998.
Count Your Eggs Before They Hatch. BEEF Magazine. Pages 8-12. April 1998.
Genetic Resistance to parasites. BEEF Magazine. page 56. September 1998.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401292 Year: 98 Project Number: 1265-32000-044-11 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

Title: EFFECTS OF TREATING CATTLE WITH ANTHELMINTICS ON IMMUNE RESPONSIVENESS AND PRODUCTION

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Gastrointestinal (GI) nematode infections cause major losses to the American cattle industry due to decreased productivity of infected animals. Current control procedures are based on the repeated administration of anthelmintic drugs to a large proportion of the cattle herd. Attempts to control parasites by pasture management are either ineffective, or are economically unfeasible. The alternative to these measures is to use the host immune system to control the intensity of parasite infection. Until recently there was little evidence that the bovine immune system was capable of limiting the magnitude of infection by GI nematodes. Recent studies by our laboratory has demonstrated that for most of the nematode species, even a short period of exposure is very effective in inducing the bovine immune system to reduce the number parasites that become established in the host. The major exception to this is Ostertagia ostertagi, but even with this species, the immune system effectively reduces parasite transmission by reducing egg output by the female worms.
These studies indicate that it is feasible to control nematode infections by using the host immune system. Before this method of control can be implemented a number of basic questions must be answered. At the present time nothing is known concerning the exact mechanism that causes the resistance to infection. Similarly, there is a lack of information concerning the ability of Ostertagia to evade an immune response that appears to function very effectively against the other nematode species. We have recently also shown that host genetics play an important role in determining if individual cattle become immune or not, but as of this date little information concerning the genes

involved is available. Finally, there is a complete lack of information available concerning the practical use of immunity-based systems into a modern production system. Information in these four areas is necessary for the integration of immunologically based control programs into a profitable and sustainable livestock management system.

2. How serious is the problem? Why does it matter?

Current estimates of the cost of GI nematodes to the American cattle industry are in the area of $2 to $8 billion dollars per year. This

Accession: 0401292 Year: 98 Project Number: 1265-32000-044-11 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

cost in based upon the cost of anthelmintic used each year, and on decreased productivity and growth in infected cattle. This easily makes GI nematodes the most costly parasitic infections of American cattle. Although the anthelmintics currently used to control the parasites are very efficacious, and extremely safe, there are increasing concerns that within a very short time period such control programs will be inadequate.

Resistance to the drugs by the parasites is increasing worldwide. Resistance to multiple classes of anthelmintics is well documented in New Zealand, Africa, and South America. This is considered such a problem that FAO of the United Nations has recently planned a very large survey of the incidence of resistant parasites in South America. In addition, there is increasing concern by consumers over the presence of drug residues in their food, and because current control programs are entirely predicated on drug administration, organic farmers are left with no means to control parasite-induced loss.

At the same time, there are major environmental changes taking place that will significantly alter parasite transmission patterns. These include loss of public grazing lands because of environmental concerns, and a movement by producers to more economical intensive grazing systems. Both of these factors will significantly increase stocking densities, and exacerbate parasite problems. Adequate parasite control will require even heavier usage of the drugs, thus increasing the selective pressure for drug-resistant parasites. Also the clearly evident warming of the environment will have drastic effects upon parasite transmission patterns in the US.

Taken as a whole, GI nematodes of cattle remain an extremely costly infections for the cattle industry. At present the availability of efficacious drugs have rendered this a chronic problem, and the economic effects are considered to be a normal expense of the livestock raising system. A number of factors could greatly exacerbate the problems posed by these parasites. The effects could range from catastrophic losses due to drug failures, to changes in geographic areas at risk.

The only feasible and economically viable approach to heavy anthelmintic usage is the use of the host immune system to control disease severity

and transmission. Because drug availability has reduced the perceived impact of the parasites, there are few laboratories left with substantial research efforts in GI nematode immunobiology. Without increased knowledge of the fundamental interaction of parasites and host immune system components, such immunity-based control programs will not be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Accession: 0401292 Year: 98 Project Number: 1265-32000-044-11 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

National Program Area 101 Animal Genomes, Germplasm, and Development (30%); National Program Area 103 Animal Health (70%).
These studies add basic and applied information concerning effective and sustainable methods to reduce parasite associated production losses. The use of host immunity in an integrated control program will improve the efficacy of cattle production systems, and result in overall healthier animals by reducing stress associated with parasitic infections, and by removing immunomodulatory pressure exerted by the parasites. In addition, identification of protective immune responses, and the mechanisms by which they function will allow the identification and manipulation of genes controlling resistance.

4. What was your most significant accomplishment this past year?

Discovered that excreted products of Ostertagia ostertagi significantly interfere with the growth of bovine T lymphocytes. This inhibition of growth is not the result of toxic activity by parasite products, but instead appears to involve the induction of inhibitory cytokines from host cells. This activity could explain why this parasite induces such strong immune responses at sites distant from the parasite, but very weak responses in the tissue immediately adjacent the parasite itself. Demonstrated that cattle raised under commercial production conditions can show different levels of cellular immune responses if they have been exposed to different levels of parasite control.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

This is the first year of this project.
Cattle become immune to almost all the GI nematodes after a relatively short exposure to the parasites. The exception is Ostertagia. Cattle can remain susceptible to this parasites for months or even years. We have demonstrated that Ostertagia releases substances that markedly retard T lymphocyte growth.

6. What do you expect to accomplish during the next year?

Studies are in progress to determine if these substances are the reason why cattle remain susceptible to Ostertagia in spite of the strong immunogenicity of the parasite. We have demonstrated that GI nematode infections can influence the magnitude of cattle immune responses arises after immunization with unrelated antigens. The potential ability of these parasites to act as stressors on the host, resulting in an increased susceptibility to other infectious agents highlights further the importance of these parasites reducing the efficiency of cattle

Accession: 0401292 Year: 98 Project Number: 1265-32000-044-11 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.2.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 30%
103 Animal Health 70%

raising systems. At the present time there are two potential mechanisms to explain this immunomodulatory activity of the parasites. The first is the extraordinary stereotypic immune response, that a particular immune protein or cytokine, termed interleukin-4 (IL4), biases the animals' immunity so that it is incapable of mounting beneficial delayed-type hypersensitivity responses. The second is the secretion of T-cell growth inhibitors by the parasites. These possibilities are currently being investigated. These studies have further confirmed the presence of different kinds of immune responses to the different parasites, and have begun to define the level of exposure necessary to elicit the responses. They have also confirmed a genetic control of immune responses, and the idea that the parasites are over dispersed in cattle herds. These studies have also resulted in a more careful description of seasonal transmission patterns for the important cattle nematodes. These studies have provided the basis for future testing of control programs based on utilization of the host immune system. Work will continue to define parasite products the inhibit the growth of T lymphocytes, and studies will be initiated to clone genes and generate gene products within larval stages of Ostertaga ostertagi to better understand its ability to attenuate the host immune responses to the parasite.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The scientist involved has answered numerous phone calls from cattle producers who have heard about the work, and has discussed in length these results with cattle individual cattle producers in Maryland, Missouri, New Hampshire, Pennsylvania, West Virginia, Virginia, and Kentucky. Presented training session to practicing Veterinarians from the Southwestern U.S. on practical parasite control in San Diego, CA in February 1998. The training session was supported by Pfizer, Animal Health as part of their program of technology transfer to field

situations.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Count Your Eggs Before They Hatch. BEEF Magazine. Pages 8-12. April 1998.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149374 Year: 98 Project Number: 1265-32000-048-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

Title: REDUCE MASTITIS INCIDENCE AND ANTIBIOTIC USE BY ENHANCING COW'S NATURAL DEFENSES

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Economic loss to the dairy industry due to mastitis can be reduced by enhancing the cow's natural defenses to bacterial infection of the mammary gland. Thus, means of improving the cow's natural defenses are urgently needed to minimize the use of antibiotics in the prevention of bacterial infection of the mammary gland. Widespread use of antibiotics has resulted in the development of resistant strains of bacteria which has necessitated periodic development of new more potent antibiotics. This poses the awesome possibility that the time is rapidly approaching when strains of bacteria will be produced that are resistant to any and all antibiotics. The use of antibiotics also poses the ever present problem of antibiotic contamination of the milk supply.
The bacteria causing the greatest loss to the dairy industry are Staphylococcus aureus and Gram-negative species. Staphylococcus aureus and gram-negative bacteria are ubiquitous in the environment and result in 85% of all cases of mastitis. More importantly once inside the gland S. aureus adheres to and penetrates the tissues lining the gland to form abscesses that are impervious to antibiotics and host defense mechanisms. This results in chronic cases of mastitis that result in culling of valuable milking stock.
Infections by Gram-negative bacteria are especially debilitating if contracted soon after calving, usually resulting in death of the cow. Death is due to the failure of the udder to control the inflammation caused by the endotoxin being produced by the rapidly multiplying coliform organisms in the milk.
Current investigations are focused on determining the nature of S. aureus virulence factors and means of shoring up the animals defenses to

combat them. Virulence factors being studied are: 1) means of entrance of the bacteria into the gland, 2) mechanism(s) of avoiding host cellular and humoral defenses and 3) mechanism(s) of adherence and penetration into the tissue. Means of shoring up the animal s defenses to combat bacterial infection of the mammary gland under current investigation include studies on: 1) strain variation in the various virulence factors, 2) strain distribution in the national herd, 3) incorporation of the predominant virulence factors in a vaccine(s), 4) methods of delivery of the vaccine(s), 5) immunomodulators to enhance the immune defenses and 6) nutritional factors effecting immune

Accession: 0149374 Year: 98 Project Number: 1265-32000-048-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

function.

2. How serious is the problem? Why does it matter?

Mastitis is the most costly of all dairy cattle diseases, resulting in losses of over $2,000,000,000 annually. Frequency of mastitis increases as milk production rises. The disease reduces milk production 5-30% per infected gland, contributing 65-70% of the total loss due to mastitis. Significant losses also occur in beef cattle, goats, sheep and pigs. Additional losses connected with mastitis include milk discarded due to antibiotic treatment, premature culling due to chronic infection, increased labor, costs of surveillance of milk supply for antibiotics and general udder health costs (including veterinary and drug expenses). In the absence of effective vaccines, present control relies heavily upon antibiotics and topical germicidal chemicals.
The dairy industry requires new tools to solve the mastitis problem. Use of antibiotics and other drugs and chemicals in the dairy industry is one of the greatest threats to food safety. Surveys indicate that at least 5% of bulk milk shipments and 30% of milk sold to consumers contains detectable amounts of antibiotics and drugs. This presents a significant human health hazard. Also, the antibiotics approved for treating mastitis are increasingly ineffective, largely due to the appearance of resistant strains. This presents the awesome danger of the development of strains of bacteria that are resistant to all current and future developed antibiotics.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%).
This research allows scientists to characterize bacterial strains according to their virulence factors and to determine their distribution in the national herd. It also results in the development of environmentally sound control measures applicable to the national dairy herd.

4. What was your most significant accomplishment this past year?

Identified three strains of S. aureus whose characteristics encompass the characteristics of 100% of the S. aureus infecting cows in the national herd. The three strains were included in a trivalent vaccine and found to produce an immune response that enhanced the ability of white blood cells (neutrophils) to engulf (phagocytose) each of the strains and to block their adherence to cells lining the mammary gland. A method for incorporating vaccine components in polymerized

Accession: 0149374 Year: 98 Project Number: 1265-32000-048-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

microspheres was developed that allowed for the production of microspheres of various sizes and release rates. Small microspheres were designed to be taken up by and rapidly release their contents to the antigen presenting cells of the immune system. The larger microspheres were designed to remain in the extracellular spaces and release their contents over an extended period of time. The two sizes of microspheres produced a rapid immune response that was sustained over an extended period of time. This will allow delivery of vaccines that will produce a sustained immune response with a single injection as compared to 3-4 injections using conventional means of vaccine delivery. This will minimize labor and record keeping and facilitate the vaccination of animals that are not handled frequently, i.e., beef cattle and sheep.

Animals with mastitis are exposed to toxins in their udders because endotoxins are excreted by the bacteria causing the infection. We discovered that udders of cows when first exposed to endotoxin produced by Gram-negative pathogens produce tumor necrosis factor (TNF), an important protein responsible for initiating the inflammatory response. However, a second exposure to endotoxin did not cause release of TNF. This important finding indicates that the udder has the capability of protecting the cow from an overwhelming inflammatory response, and possible death, by blocking release of TNF after a first time exposure to endotoxin. Discovering the mediators of this down-regulation could have important implications in the treatment and prevention of coliform mastitis.

Cloned the gene for producing soluble CD14. Soluble CD14 binds and neutralizes endotoxin which can be fatal to cows during the early periparturient period. Cloning of this protein allows for the production and use CD14 in the treatment of clinical mastitis caused by Gram-negative bacteria. Currently there is nothing available for neutralizing endotoxin. Produced anti-bovine neutrophil monoclonal antibodies that enhance neutrophil function. These antibodies were linked to monoclonal antibodies to mastitis pathogens for the production of the world's first bispecific antibodies for the targeted lysis of mastitis pathogens in vivo. This discovery provides an alternative to

antibiotics in the prevention and treatment of mastitis. Development of bispecific antibodies is being supported by industry.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Produced the first monoclonal antibodies to bovine immunoglobulin isotypes (proteins that act as antibodies to specific antigens) and the first bovine/mouse monoclonal hybrids that produced bovine immunoglobulin isotypes. These pure bovine immunoglobulins have been

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NATL PROG(S) 103 Animal Health 100%

used as standards and in sequencing studies to characterize and compare their structure with other species. These reagents opened the door for numerous in-depth studies of the bovine immune system in various laboratories throughout the world. It was approximately 15 years before comparable reagents were available commercially. Using these antisera, our laboratory characterized major areas of the bovine mammary gland immune system. Characterized changes that occur on the surface of S. aureus upon entering the mammary gland, namely the formation of a polysaccharide capsule covering the organism, that protects the organism from the host s defense mechanisms. Developed immunization protocols that produced antibodies to the capsule that decreased the effectiveness of the capsule as a virulence factor. Produced the first monoclonal antibodies to subpopulations of bovine neutrophils and used these to identify subpopulations that migrate from blood into the mammary gland and their relative ability to phagocytose and kill bacteria.
Developed the first cell culture system consisting of a layer of endothelial cells (cells that line the blood vessels), a layer of fibroblast embedded in collagen (representing the interstitial spaces) and a layer of epithelial cells (cells that line the inside of the teat, the milk ducts and secretory alveoli) supported on a permeable matrix. This in vitro system mimics the blood/milk barrier of the mammary gland and allowed the study of passage of blood components into the milk and vice versa and for the study of bacterial interaction with cells lining the mammary gland. Developed a trivalent S. aureus vaccine that represents the surface characteristics of 100% of the strains present in the national herd. Incorporated this vaccine into polymeric microspheres of various sizes and release rates that resulted in a vaccine and delivery system that produced a long lasting immune response with a single injection.
Research in this project focused world-wide attention on the errors involved in the determination of somatic cell counts in cow and goat milk. These counts form the basis of national and international abnormal milk control programs which determines the price dairymen receive for milk. Reliable stains and accurate counting procedures for cells in milk were developed that are industry standards and currently

used by regulatory agencies the world over. It was further established that neither physiological or environmental stressors caused alterations in the somatic cell content of milk. As a consequence, dairymen, leaders of industry and researchers throughout the world have accepted milk somatic cell counts as an index of milk quality.

In vitro procedures for measuring neutrophil function and procedures for the isolation of white blood cells from blood and milk were developed, which are used by researchers throughout the world. The discovery that neutrophil function is depressed after parturition, a time of increased susceptibility of the gland to mastitis, stimulated industry interest in

Accession: 0149374 Year: 98 Project Number: 1265-32000-048-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

the use of cytokines as modulators of neutrophil function during the periparturent period. Were the first to demonstrate that differences existed among cows in the ability of neutrophils to ingest and kill bacteria. Further showed a relationship between neutrophil function and susceptibility to mastitis. These differences in phagocytic competence may provide methods of capitalizing on genetic differences in natural resistance, which could be quantified at an early age.

6. What do you expect to accomplish during the next year?

During the next year we will test vaccines for mastitis. We will compare 1)the trivalent S. aureus vaccine in the form of whole cells with 2)purified components of the three organisms in microspheres. We will test efficacy of the vaccines by determining their ability to produce an effective immune (antibody and cell mediated) response, using a phagocytosis in vitro assay and an adherence assay using monolayers of mammary epithelial cells. We will also test the therapeutic effect of the three bacterial organism vaccine, along with antibiotics, on established S. aureus infections in collaboration with scientists at Michigan State University.
Field studies will be initiated to evaluate use of bifunctional antibodies in the treatment of infections caused by S. aureus.
Monoclonal antibodies to CD14 will be produced and an ELISA will be developed for quantitating this protein in blood and milk. Studies on the interrelationships of this protein with endotoxin and TNF will be initiated to better understand the immunopathology of infections caused by Gram-negative bacteria.
A cell death, or apoptosis, assay will be developed to quantitate viability of neutrophils after they enter the mammary gland. This will provide new information on the ability of neutrophils to defend the mammary gland against infections by mastitis pathogens.
We will initiate studies on production of immune stimulatory proteins, termed cytokines, by mammary epithelial cells, endothelial cells and neutrophils. This will provide information on the pivotal role played by cytokines in resolving bacterial infections in the mammary gland.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Polyclonal and monoclonal antibodies to bovine immunoglobulins were produced, prior to any available commercially, and supplied to researchers throughout the US and abroad. These were recognized as the highest quality available after they became commercially available.

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Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

Developed the first in vitro model of the blood/milk barrier - a culture of cells lining the blood vessels and cells lining the mammary gland separated by interstitial cells and proteins characteristic of the interstitial tissues in the mammary gland.
Supplied cells and instructed other laboratories in the development of the model for various studies. A phenolic teat dip was developed and shown to be effective in preventing new intramammary infections. This teat dip will be made available to dairymen. We have shown that treatment of beef cows at weaning by intramammary infusion of antibiotics will reduce incidence of mastitis and improve calf weaning weights. Adoption of this technology by beef cattle producers will increase profits of cattle producers.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Coupled antibodies fend off mastitis. Agriculture Research, June 1998. pp17.
Milk money. The Cattleman, June 1998, pp102-108.

PUBLICATIONS:

01.
GUIDRY, A.J., FATTOM, A., PATEL, A. and OBRIEN, C. 1997. Prevalence of capsular serotypes among Staphylococcus aureus isolates from mastitic cows in the United States. Vet. Microbiol. 59:53-58.

02.
GUIDRY, A.J. and OBRIEN, C.N. 1997. Current awareness of bovine mammary gland immunology. Flemish Vet. J. Suppl. 66:341-358.

03.
GUIDRY, A.J. and OBRIEN, C.N. 1998. A Bovine mammary endothelial/epithelial cell culture model of the blood/milk barrier.

Can. J. Vet. Res. 62:117-121.

04.
BURVENICH, C., PAAPE, M.J. and GUIDRY, A.J. 1998. Possible regulatory role of somatotrope hormone (growth hormone) on the inflammatory reaction and defence ... gland. Domestic Animal Endo. pp. 1-24.

05.
SMITS, E., BURVENICH, C., GUIDRY, A.J. and ROETS, E. 1998. In vitro expression of adhesion receptors and diapedesis by polymorphonuclear neutrophils during ... mastitis. Infect. Immun. 66:6:2529-2534.

Publications: (Continued)

06.
SMITS, E., BURVENICH, C., GUIDRY, A.J., CIFRIAN, E. and RAINARD, P. 1998. An in vitro approach to bovine mastitis. A half century of lactation biology ... research. Flemish Vet. J. Suppl. 66:315-339.

07.
GUIDRY, A.J., FATTOM, A., PATEL, A., OBRIEN, C.N., SHEPHERD, S. and LOHUIS, J. 1998. A serotyping scheme for Staphylococcus aureus isolated from cows with mastitis. Am. J. Vet. Res. 59:12:1537-1539.

08.
DOSOGNE, H., CAPUCO, A.V., PAAPE, M.J. and BURVENICH, C. 1998. Acyloxyacyl hydrolase activity in circulating neutrophils from cows around parturition. J. Dairy Sci. 81:672-677.

09.
DOSOGNE, H., BURVENICH, C. and PAAPE, M.J. 1998. Effect of extracellular Ca2+ and Mg2+ on opsonic and non-opsonic phagocytosis of Escherichia coli by bovine circulating neutrophils. Comp. Haematology Int. 8:25-28.

10.
RAINARD, P., SARRADIN, P. PAAPE, M.J. and POUTREL, B. 1998. Quantification of C5a/C5adesArg in bovine plasma, serum and milk. Vet. Res. 29:73-88.

11.
TALBOT, N.C., PAAPE, M.J. and WORKU, M. 1998. Selective expansion and continuous culture of macrophages from pig blood. Vet. Immuno. Immunopath. 64:173-190.

12.
BROWN, M.A., DUENAS, I., PAAPE, M.J., JACKSON, W.G., MIESNER, J.R. and BROWN, A.H. 1998. Evaluation of mastitis-causing organisms in Angus, Brahman, and ... Bermudagrass. Professional Animal Sci. 14:127-132.

13.
CONTRERAS, A., PAAPE, M.J. and MILLER, R.H. 1998. Prevalence of Staphylococcus epidermidis intramammary infection in a commercial dairy goat herd. Small Rumin. Res. 25:110-115.

14.
BURVENICH, C., DOSOGNE, H., HOEBEN, D., GUIDRY, A.J. and PAAPE, M.J. 1998. Immune mechanisms in the bovine lactating udder. Congr's organis' par la Soci't' Francaise de Buiatrie, Paris, France. pp. 256-272.

Approved: D.F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400874 Year: 98 Project Number: 1265-32000-048-02 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

Title: IMMUNIZATION WITH STAPHYLOCOCCUS AUREUS ANTIGENS INCORPORATED INTO MICROSPHERES

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Economic loss to the dairy industry due to mastitis can be reduced by enhancing the cow's natural defenses to bacterial infection of the mammary gland. Means of enhancing the cow s natural defenses are urgently needed to minimize the use of antibiotics in the prevention of bacterial infection of the mammary gland. Widespread use of antibiotics has resulted in the development of resistant strains of bacteria which has necessitated periodic development of new more potent antibiotics. This poses the awesome possibility that the time is rapidly approaching when strains of bacteria will be produced that are resistant to any and all antibiotics. The use of antibiotics also poses the ever present problem of antibiotic contamination of the milk supply.
The bacteria causing the greatest loss to the dairy industry are Staphylococcus aureus. Staphylococcus aureus bacteria are ubiquitous in the environment and result in 35% of all cases of mastitis. More importantly once inside the gland S. aureus adheres to and penetrates the tissues lining the gland to form abscesses that are impervious to antibiotics and host defense mechanisms. This results in chronic cases of mastitis that result in culling of valuable milking stock.
Current investigations are focused on determining the nature of S. aureus virulence factors and means of shoring up the animals defenses to combat them. Virulence factors being studied are: 1) means of entrance of the bacteria into the gland, 2) mechanism(s) of avoiding host cellular and humoral defenses and 3) mechanism(s) of adherence and penetration into the tissue. Means of shoring up the animal's defenses to combat bacterial infection of the mammary gland under current investigation include studies on: 1) strain variation in the various

virulence factors, 2) strain distribution in the national herd, 3) incorporation of the predominant virulence factors in a vaccine(s), 4) methods of delivery of the vaccine(s), 5) immunomodulators to enhance the immune defenses and 6) nutritional factors effecting immune function.

2. How serious is the problem? Why does it matter?

Mastitis is the most costly of all dairy cattle diseases, resulting in losses of over $2,000,000,000 annually. Frequency of mastitis increases

Accession: 0400874 Year: 98 Project Number: 1265-32000-048-02 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

as milk production rises. The disease reduces milk production 5-30% per infected gland, contributing 65-70% of the total loss due to mastitis. Significant losses also occur in beef cattle, goats, sheep and pigs. Additional losses connected with mastitis include milk discarded due to antibiotic treatment, premature culling due to chronic infection, increased labor, costs of surveillance of milk supply for antibiotics and general udder health costs (including veterinary and drug expenses). In the absence of effective vaccines, present control relies heavily upon antibiotics and topical germicidal chemicals.
The dairy industry requires new tools to solve the mastitis problem. Use of antibiotics and other drugs and chemicals in the dairy industry is one of the greatest threats to food safety. Surveys indicate that at least 5% of bulk milk shipments and 30% of milk sold to consumers contains detectable amounts of antibiotics and drugs. This presents a significant human health hazard. Also, the antibiotics approved for treating mastitis are increasingly ineffective, largely due to the appearance of resistant strains. This presents the awesome danger of the development of strains of bacteria that are resistant to all current and future developed antibiotics.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%)
This research is an integral part of a larger program to develop means of immunizing cows against mastitis pathogens in order to minimize the use of antibiotics. This will result in the development of a more environmentally sound means of control for mastitis in the national herd.

4. What was your most significant accomplishment this past year?

Cows were immunized with S. aureus in the form of a conventional vaccine and in the form of a slow release vaccine. Both vaccines were given with an proprietary immunostimulant supplied by Ajinomoto. The

conventional vaccine was superior to the slow release vaccine and the immunostimulant supplied by Ajinomoto had no effect.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Cows were immunized with S. aureus in the form of a conventional vaccine and in the form of a slow release vaccine. Both vaccines were given with an proprietary immunostimulant supplied by Ajinomoto. The conventional vaccine was superior to the slow release vaccine and the

Accession: 0400874 Year: 98 Project Number: 1265-32000-048-02 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

immunostimulant supplied by Ajinomoto had no effect.

6. What do you expect to accomplish during the next year?

The project report will be written and submitted to Ajinomoto. The project will be terminated as of July 1999.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The results showed that the current vaccine formulation was good.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401049 Year: 98 Project Number: 1265-32000-048-03 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

Title: METHODS OF CONTROLLING UDDER INFLAMMATION

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Economic loss to the dairy industry due to mastitis can be reduced by enhancing the cow's natural defenses to bacterial infection of the mammary gland. This is urgently needed to minimize the use of antibiotics in the prevention of bacterial infection of the mammary gland. Widespread use of antibiotics has resulted in the development of resistant strains of bacteria which has necessitated periodic development of new, more potent antibiotics. This poses the awesome possibility that the time is rapidly approaching when strains of bacteria will be produced that are resistant to any and all antibiotics. The use of antibiotics also poses the ever present problem of antibiotic contamination of the milk supply.
Current investigations are focused on determining: 1)if intramammary infusion of a natural product, arginine, will eliminate subclinical and clinical mastitis, and 2)if a product from pineapples, bromelain, fed as a dietary supplement will increase efficiency of mammary leukocytes to kill bacteria resulting in reduced leukocyte counts in milk.

2. How serious is the problem? Why does it matter?

Mastitis is the most costly of all dairy cattle diseases, resulting in losses of over $2,000,000,000 annually. Frequency of mastitis increases as milk production rises. The disease reduces milk production 5-30% per infected gland, contributing 65-70% of the total loss due to mastitis. Significant losses also occur in beef cattle, goats, sheep and pigs. Additional losses connected with mastitis include milk discarded due to antibiotic treatment, premature culling due to chronic infection,

increased labor, costs of surveillance of milk supply for antibiotics and general udder health costs (including veterinary and drug expenses). In the absence of effective vaccines, present control relies heavily upon antibiotics and topical germicidal chemicals.

The dairy industry requires new tools to solve the mastitis problem. Use of antibiotics and other drugs and chemicals in the dairy industry is one of the greatest threats to food safety. Surveys indicate that at least 5% of bulk milk shipments and 30% of milk sold to consumers contains detectable amounts of antibiotics and drugs. This presents a significant human health hazard. Also, the antibiotics approved for

Accession: 0401049 Year: 98 Project Number: 1265-32000-048-03 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

treating mastitis are increasingly ineffective, largely due to the appearance of resistant strains. This presents the awesome danger of the development of strains of bacteria that are resistant to all current and future developed antibiotics.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%)
This research allows scientists to develop environmentally sound control measures applicable to the national dairy herd.

4. What was your most significant accomplishment this past year?

A new nonantibiotic treatment for mastitis using the amino acid arginine was developed. Argenine enhanced the killing of bacteria by neutrophils, a type of white blood cell that protects the body from infection. Further testing in the test tube an din the udders of cows demonstrated that argenine was not irritating to mammary tissues. Bromelain, an anti-inflammatory product made from pineapples, when fed to cows reduced the somatic cell count in milk. Also, the milk somatic cell count in milk increased during periods of hot weather for control cows but not for cows fed bromelain.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Arginine increased functional ability of neutrophils and was not irritating to mammary tissue. Intramammary infusion of arginine during periods of clinical mastitis may prove to be a more effective alternative to antibiotics in eliminating clinical mastitis. The feeding of bromelain to dairy cows reduced leukocyte counts in milk. Because the leukocyte count in milk is used as the basis of national and international abnormal milk control programs which determines the price dairymen receive for milk, reduction of leukocyte count by feeding

bromelain could result in large economic returns to dairy producers.

6. What do you expect to accomplish during the next year?

During the next year we will infuse arginine into mammary glands of lactating cows during experimentally induced and natural cases of clinical mastitis. Cure rates will be compared to noninfused and antibiotic infused controls.
Field studies will be initiated to determine effects of bromelain on reducing somatic cell counts in the milk of goats and cows. Studies

Accession: 0401049 Year: 98 Project Number: 1265-32000-048-03 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 100%
NATL PROG(S) 103 Animal Health 100%

will be conducted in Maryland dairy herds and goat herds in Spain.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Bromelain will be made available to dairy and goat producers within two years to reduce cell counts in milk. There are no known constraints. Use of arginine as a intramammary infusion product for the treatment of mastitis will become available within the next three years. Arginine must receive FDA approval before use. This will require efficacy studies comparing arginine with a commonly used antibiotic currently used for treating clinical mastitis.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149650 Year: 98 Project Number: 1265-32000-049-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

Title: STRATEGIES TO CONTROL SWINE PARASITES AFFECTING FOOD SAFETY

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The prevalence of immunosuppressive therapies for transplantation and control of malignancies and viral diseases, along with the aging of the general population, reduces immune competence and increases the human risk of infection by foodborne pathogens. Swine have two prominent foodborne parasitic agents, the nematode muscle worm, Trichinella spiralis, and the tissue protozoan, Toxoplasma gondii. In addition, eggs from the large roundworm, Ascaris suum, represent an emerging disease where dispersal of pig manure for agricultural uses are increasing the contact between edible fruits and vegetables and infectious eggs in the manure. Outbreaks of human infection could compromise the impression of pork as a safe source of nutritious edible tissue and reduces consumption nationally and lowers exports. No drugs are available to cure infections of T. spiralis or T. gondii, and drug control for A. suum is effective but inefficient. All of these infections can be regulated by proper herd management, but there is a large capital investment and breaches in protocol increase risk to consumers. In addition, the cost of development of new drugs has suppressed commercial research and development reducing the arsenal of replacement drugs to control the anticipated rise in drug resistance. Further, secondary microbial diseases that follow heavy worm infections threaten to increase the levels of another foodborne pathogen, campylobacter, in infected herds.
The current approach to control these infections is based on enhanced swine innate and acquired immunity and vaccine development. This is a long term strategy because basic information on the intricacies of the immune system in swine necessary for effective immunological controls is

lacking. The development of information on the swine immune system is increasing as cooperation between university, industry and government laboratories merges. The eventual application of this information will benefit not only the control of parasitic infections, but also control of viral and microbial infections because of the intricate balance of responses inherent in a robust immune responses. Efforts are directed at molecular regulators of immunity, receptor biology, innate protective mechanisms, cellular immunity and antibody responses with studies targeted at he intestinal mucosal immune system of pigs. These studies will reduce the threat of foodborne infection and enhance animal health

Accession: 0149650 Year: 98 Project Number: 1265-32000-049-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

to lower the requirement for antibiotics to control infectious diseases.

2. How serious is the problem? Why does it matter?

Outbreaks of human trichinellosis are sporadic and limited to peculiar dietary habits and poor herd management of small farms, but infection of swine with roundworms is ubiquitous. Surveys of the prevalence of toxoplasmosis indicate a high level of herd infection in major swine producing areas and an increase in seroprevalence in humans in the U.S. and world wide. Proof of human toxoplasmosis from eating pork products is tenuous, but the association with high prevalence of infection in commercial herds increases suspicions and represents an undesirable publicity threat to the industry. The reputation of pork as the "other white meat" has been carefully promoted over the last decade to change public opinion about the image of pork as "wormy" or requiring greater household care. This effort could suffer a dramatic turn if pro-active procedures to control sources of parasitic infections are not vigorously pursued. Similarly, an awareness of outbreaks of human infection with the eggs of A. suum in Japan and Holland portend such infections in the U.S., especially in high pig production areas where manure management and field and ground water contamination with microbes from swine manure are a public health issue. A readiness to anticipate a potential problem from inadequate manure management requires improved control procedures. Vaccination against gastrointestinal worms would complement existing control strategies through procedures that are familiar and acceptable to the public and would provide long term animal resistance.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 108 Food Safety (70%); National Program Area 103 Animal Health (30%).
This research fits into an overall strategy to reduce the potential for infection of swine with parasitic organisms that could infect humans through the ingestion of contaminated tissues. Studies on the basic

mechanism of immunological control of infectious diseases of swine supports development of vaccines against parasitic, viral and microbial infections that compromise animal health and productivity.

4. What was your most significant accomplishment this past year?

Development of reagents to study changes in porcine immunity, specifically means to measure immune regulator (cytokine) gene transcription and protein synthesis. These are significant because of their requirement for characterization of swine immunity to infectious

Accession: 0149650 Year: 98 Project Number: 1265-32000-049-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

agents. Assays have been developed that can now measure several porcine cytokines including interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12 IL-12 receptor, IL-13, IL-15 and interferon-gamma (IFNg), as well as protein detection assays for IFNg. These reagents can be used to measure responses to parasitic, microbial and viral pathogens, and provide important information for vaccine development. These procedures have wide applicability to porcine immune system research both nationally and internationally.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Over the last 3 years molecular markers of distinct Trichinella genotypes have been used to identify strains that have enhanced natural resistance to freezing. Freezing edible tissues has been a major commercial and consumer-based control strategy of decontamination of potentially infectious edible tissue. Introduction of freeze-resistant strains of Trichinella into the food chain could have severe negative impact on existing control strategies. These molecular markers could insure that these strains will not go undetected.
Studies of the immunobiology of toxoplasmosis in swine have demonstrated that strong protective immunity can be induced by irradiated oocyts and attenuated strains, but immunity is not sterilizing and the threat of low level infections remains. Novel procedures for enhancing the porcine immune system will require additional information on swine immunomodulators.
Basic information on the swine immune system has increased dramatically with the development of reagents that characterize swine lymphocytes, molecular probes for detecting peptide hormone regulators of immunity (cytokines), and cell separation procedures for isolating cells from intestinal mucosal surfaces. This information has been used to evaluate unique aspects of the swine immune system. The availability of these reagents has enabled us to better elucidate the host immune responses to gastrointestinal nematodes as well as to common protozoan infections such as T. gondii. The list of reagents includes probes for

interleukins IL-2, IL-4, IL-5, IL-10, IL-12, IL-12 receptor, IL-13, IL-15, and INF-gamma. In addition, we have cloned and expressed genetically engineered interleukin IL-12 permitting us to initiate a study on the influence this cytokine has on attenuating nematode and protozoal infections in swine hosts. The impact of these reagents on the genetic changes corresponding to immunity and susceptibility of infected pigs will be invaluable in the development of novel control strategies. This has already led to a clear demonstration that a strong anti-parasitic response to infection reduces the level of immunity to microbial agents that produce secondary disease. This important observation supports the

Accession: 0149650 Year: 98 Project Number: 1265-32000-049-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

idea that maintenance of higher levels of herd health against parasitic infections will minimize the risk of inadvertent and uncontrolled secondary infections without the use of antibiotics. Further, the reagents developed have been used to study swine immunity to a variety of viral and bacterial agents by independent investigators throughout the world.

6. What do you expect to accomplish during the next year?

During the next year recombinant porcine IL-12 that was characterized and synthesized in our laboratory through an external funding consortium and will be administered to neonatal swine to prophylactically control disease caused by parasitic and microbial infections. These studies will demonstrate whether a exogenous natural cytokine can substitute for the growth promoting and resistance enhancing properties of antibiotics. The success of this approach would have a major effect on minimizing the use of antibiotics in early pig weaning strategies. This approach is based primarily on our extended development of probes and reagents to study changes in host immune responses to parasitic infections. Additional cytokine gene expression probes and expanded development of antibody assays for IL-4 will also help characterize the dynamic response of pigs to both parasitic and microbial pathogens. Agonists of the IL-4 receptor will be developed to demonstrate whether stimulation of the IL-4 receptor in pigs will induce rapid expulsion of gastrointestinal worms in pigs in a manner similar to that described in experimental rodent models.
Collaborative studies with scientists at the Michigan State University School of Veterinary Medicine will also apply these studies to a model developed in Beltsville to examine secondary infection of pigs with Campylobacter jejunii. Further work on Trichinella will address the new born larval stage to understand the early events in the infection process and identify unique molecular targets for eliminating the infection in suspect pigs. Molecular probes to differentiate distinct Trichinella genotypes will be described in a plan to initiate patent protection of the technology.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Reagents generated to study immune responses in swine have been made available to the research community and molecular probes and primers appear in GenBank. Panels of monoclonal antibody reagents have been transferred to several commercial sources. To date, numerous requests

Accession: 0149650 Year: 98 Project Number: 1265-32000-049-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

for oligonucleotide probes, primers, monoclonal antibody reagents to identify lymphocyte subpopulations have been provided to both national and international scientists. This stimulates communication and interactions that enhance the strength of the scientific community to address agricultural problems of national interest.
The cloning of a functional IL-12 cytokine has lead to corporate interest and involvement in synthesizing this reagent and making it available for research and commercial use following a thorough and ongoing investigation at the USDA of its application as a general immune simulator to attenuate certain classes of infectious agents of swine. Constraints to the application of these technologies i.e. genetically synthesized cytokines, are related to the present lack of information on the effectiveness of their use as general immune simulators for attenuating parasitic infections. A Material Transfer Agreement has been signed with Genetics Institute, Cambridge, MA, to test human and mouse reagents for IL-13 and the IL-13 receptor in pigs.
The diagnostic tests for differentiating Trichinella will be published and will become available to the scientific and commercial communities. Methodology is presently being developed for commercializing the test to rapidly and unequivocally diagnose Trichinella genotypes. Constraints related to the development and application of tests for diagnosing parasitic diseases of swine are related to end-user acceptance of the technology rather than the effectiveness of the tests already developed or presently under development.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

HARTSOCK, T. 1998. Pig Tales. Take a look in your backyard. The Delmarva Farmer. Section 3. p3.
LUNNEY, J.K. and ZARLENGA, D.S. 1997. Detecting foodborne parasites. National Hog Farmer. pp15-16.

PUBLICATIONS:

01.
ZARLENGA, D. 1998. cDNA cloning and the construction of recombinant DNA. pp. 353-382 IN: J.J. Greene and V.R. Rao (eds.) Recombinant DNA principles and Methodologies. Marcel Decker, Inc., New York, NY.

02.
SAALMUELLER, A. and LUNNEY, J. 1998. Second international workshop on swine leukocyte differentiation antigens. Special Issue: Vet. Immunol. Immunopathol. 60:205-446.

03.
ZARLENGA, D. 1998. Molecular aspects of Trichinella speciation. Trichinellosis 9:43-46.

Publications: (Continued)

04.
ZARLENGA, D., ASCHENBRENNER, R. and LICHTENFELS, J. 1998. Genetic differences among populations of Trichinella pseudospiralis exhibited by polymorphism within ribosomal ... repeats. Trichinellosis 9:47-52.

05.
MANSFIELD, L., URBAN, J., HOLLEY-SHANKS, R., MURTAUGH, M., ZARLENGA, D., FOSS, D., CANALS, A., GAUSE, W. and LUNNEY, J. 1998. Construction of ... swine. Vet. Immunol. Immunopathol. 65:63-74.

06.
MATEU de ANTONIO, E., HUSMANN, R., HANSEN, R., LUNNEY, J., STROM, D., MARTIN, S.L. and ZUCKERMANN, F. 1998. Quantitative detection of porcine ... antigen. Vet. Immunol. Immunopathol. 61:265-277.

07.
PESCOVITZ, M.D, BOOK, B.K., LUNNEY, J.K., BOYD, P.C. and SAALMUELLER, A., et al. 1998. Summary of workshop findings for antibodies reacting with porcine T-cells. CD Workshop. Vet. Immunol. Immunopathol. 60:251-260.

08.
PESCOVITZ, M.D, BOOK, B.K., LUNNEY, J.K., BODY, P.C. and SAALMUELLER, A., et al. 1998. Analyses of monoclonal antibodies reacting with porcine CD3. ... Workshop. Vet. Immunol. Immunopathol. 60:261-268.

09.
URBAN, J., NOBEN-TRAUTH, N., DONALDSON, D., MADDEN, K., MORRIS, S., COLLINS, M. and FINKELMAN, F. 1998. Both IL4 and IL13 mediate the expulsion of a gastrointestinal ... mechanism. Immunity 8:255-264.

10.
RHOADS, M., FETTERER, R. and URBAN, J. 1997. Secretion of an aminopeptidase during transition from 3rd-4th stage larvae of Ascaris suum. J. Parasitol. 83:780-784.

11.
GAUSE, W., MITRO, V., VIA, C., LINSLEY, P., URBAN, J. and GREENWALD, R. 1997. Do effector and memory T helper cells also need B7 ligand costimulatory signals? J. Immunol. 159:1055-1058.

12.
RHOADS, M., FETTERER, R. and URBAN, J. 1998. Effect of protease class specific inhibitor on in vitro development of L3 to L4 of Ascaris suum. J. Parasitol. 84:686-690.

13.
MANSFIELD, L., HILL, D. and URBAN, J. 1997. Lymphoglandular complexes process antigen ... swine. pp. 185-195. IN: G.T. Keusch & M. Kawakami (eds.) Cytokines, cholera, & the gut. IOS press, Ohmaha, NE.

14.
ZUCKERMANN, F.A., PEAVEY, C., SCHNITZLEIN, W.M. and LUNNEY, J.K. 1998. Definition of the specificity of monoclonal antibodies against porcine CD45 and CD45R. Workshop Vet. Immunol. Immunopathol. 60:367-388.

Publications: (Continued)

15.
ZUCKERMANN, F.A., PESCOVITZ, M.D. AASTED, B., DOMINGUEZ, J. TREBICHAVSKY, I., LUNNEY, J.K., et al. 1998. Report on the ... CD8. 2nd Intnl. Swine CD Workshop. Vet. Immunol. Immunopathol. 60:291-304.

16.
SAALMUELLER, A., PAULY, T., LUNNEY, J.K., BOYD, P.C., et al. 1998. Overview of the second international workshop to define swine leukocyte cluster of ... antigens. Vet. Immunol. Immunopathol. 60:207-228.

17.
PESCOVITZ, M.D. PABST, R., ROTHKOTTER, H.J., LUNNEY, J., et al. 1998. Immunology of the pig, pp. 373-419. IN: P.-P. Pastoret, P. Griebel, et al. (eds.) Handbook ... immunology. Academic Press, London, England.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400962 Year: 98 Project Number: 1265-32000-049-07 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

Title: PREVENTION OF TOXOPLAMSA GONDII INFECTION IN PIGS

Period Covered From: 01/98 To: 09/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Major strategies for prevention of the foodborne parasitic infection of pigs, toxoplasmosis, in swine include the identification of resistance factors, and the development of vaccines with specific Toxoplasma gondii antigens to induce protective responses. This National Pork Producer's Council grant was funded to develop more knowledge about immune responses that would prevent or control T. gondii infections. Despite the extensive literature on immunity in mice and humans, little was known of swine immune responses to this infection. Antibody titers have been reported for swine but these are highly variable and, as with other species, not predictive of effective immunity. There is currently no effective drug therapy or vaccine to prevent T. gondii infection in pigs. Moreover, epidemiological studies have not been able to fully define whether management procedures can totally prevent this infection. Thus, the pig constitutes a continuos threat to public health, since T. gondii tissue cysts have been identified in commercial cuts of pork. Thus, these studies showed 1)that vaccination with treated parasites could partially protect pigs from the natural infection, 2)that pigs which are genetically resistant to this foodborne infection could be identified, and 3)that certain immune modulators, specifically interferon-gamma, are key regulators of swine responses against this parasitic infection. Thus alternate methods of controlling this foodborne infection have been identified.

2. How serious is the problem? Why does it matter?

Toxoplasmosis occurs in a variety of livestock and in humans, causing

abortions, mental retardation in newborns and encephalitis in immunocompromised individuals such as transplant recipients and AIDS and cancer patients. Among food animals, pigs are considered the major source of T. gondii infection in the U.S. Recent field studies indicate that about 3% of market pigs and 18% of breeding stock of pigs have antibodies to T. gondii. T. gondii infection in swine creates a public health concern because this parasite can be transmitted to humans through the handling and consumption of raw or undercooked meat. Although under natural conditions toxoplasmosis is not considered a common cause of clinical disease in swine, extensive epidemiological

Accession: 0400962 Year: 98 Project Number: 1265-32000-049-07 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

studies have indicated that the subclinical infection is more common. Current control methods rely heavily, if not exclusively, on management with variable results. Improved management practices for toxoplasmosis control at the farm level, such as the use of rodenticides instead of cats and intensive confinement of animals, probably explained the reduction in disease prevalence, from 24.2% in the early 80s to 3.1 % in the 90Æs, in the last decade in market pigs. However, these management procedures have not been effective in totally preventing this infection or in substantially reducing the prevalence in older breeding animals. The latest regional surveys in Iowa and Illinois indicate prevalence in this population varies from 2.9% to 17 % depending on the type of facility. Thus, alternate control strategies must be developed.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 108 Food Safety (70%): National Program Area 103 Animal Health (30%)
This research fits into an overall strategy to reduce the potential for infection of swine with parasitic organisms that could infect humans through the ingestion of contaminated tissues. Studies on the basic mechanism of immunological control of infectious diseases of swine supports development of vaccines against parasitic, viral and microbial infections that compromise animal health.

4. What was your most significant accomplishment this past year?

When genetically defined pigs were given a direct challenge with T. gondii oocysts they exhibited natural protective immunity against toxoplasmosis. Among subsets of genetically defined (swine leukocyte antigen or SLA inbred) miniature pigs it was shown that some pigs were able to respond with higher production of the immune regulator, interferon-gamma (IFNg), and had a lower T. gondii burden in their tissues, as compared with other pigs. There were also two fully resistant pigs, i.e., there was no infectious T. gondii recovered from

their tissues. Taken together, these findings suggest that, as in other species, IFNg may be the key cytokine in protection against T. gondii infection in pigs. Moreover, there may be some natural resistance factors in certain genetic types of pigs that would be associated with increased resistance to T. gondii infection. These possibilities will require further study.

More recent work, has been encouraging, since it was demonstrated that among different SLA inbred miniature pigs , SLAdd haplotype pigs were able to respond with a higher IFN-g production, lower T. gondii yield from their tissues and in one of 10 pigs a total absence of cysts after

Accession: 0400962 Year: 98 Project Number: 1265-32000-049-07 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

a successful infection with the parasite, since specific antibodies were present (8).
Taking together, these findings suggest that, as in other species, IFN-g may be the key cytokine in the protection against this infection in pigs and that at the same time there could be some potential genetically encoded resistance factors in some pigs that would be associated with increased resistance to T. gondii infection.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Information on factors that stimulate protective immune responses in pigs preventing T. gondii infections should help in the production of reliable sources of Toxoplasma free animals. To define the protective immune response of pigs to T. gondii infection, we studied vaccination protocols to completely prevent this infection in outbred and inbred pigs by feeding T. gondii oocysts inactivated by irradiation. Results from the vaccine study showed that use of the irradiated oocyst vaccine clearly induces protective immunity to challenge infection. However, it is not completely protective, because some parasite can still be recovered in tissues from the vaccinated pigs. Thus, since a single tissue cyst may be enough to induce infection in humans, it was considered that the irradiated oocyst vaccine was not completely effective in preventing this disease in swine. Because of the incomplete protection with the irradiated oocyst vaccine, future vaccine trials will have to look at more effective means of inducing protection using other vaccine sources and/or cloned antigens.
Overall, this work should help develop our understanding of the relationship between resistance, protective immunity and infection with T. gondii. Based on these research, new studies involving search of resistance genes could be designed to increase resistance in outbred pigs so pork can be produced completely T. gondii free and their meat products safe for public consumption.

6. What do you expect to accomplish during the next year?

This project has been completed.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

This research was funded by a National Pork Producer's Council grant and all results have been shared with the pork producers.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400962 Year: 98 Project Number: 1265-32000-049-07 R
Mode Code: 1265-20-00 STP Codes: 3.2.1.4 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

LUNNEY, J.K. and ZARLENGA, D.S. 1997. Detecting Food-borne parasites. National Hog Farmer. Dec. 15, 1997. pp.15-16.
HARTSOCK, T. 1998. Pig Tales. Take a look in your backyard. The Delmarva Farmer, Section 3, p.3.

PUBLICATIONS:
01.
DUBEY, J.P., LUNNEY, J.K., SHEN, S.K. and KWOK, O.C.H. 1998. Immunity to toxoplasmosis in pigs fed irradiated Toxoplasma gondii oocysts to pigs. J. Parasitol. 84:749-752.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401466 Year: 98 Project Number: 1265-32000-049-09 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

Title: CYTOKINE INDUCED IMMUNE MODULATION OF MUCOSAL RESPONSES IN NEONATAL SWINE

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Swine disease control is most important during the first months of a pig's life due to the lack of immune system development in the newborn pig. Moreover there is rapid exposure of the newborn to infectious agents when it moves from the birthing (farrowing) unit into nursery facilities that house litters from numerous sows. This Biotechnology Research and Development Corporation (BRDC) grant was established to develop new methods to stimulate neonatal swine immunity. We will assess whether administration of exogenous cytokines enhances neonatal immunity and disease responses, and determine whether this will help protect young pigs from infections and stress-induced immune deficiencies. Our studies will be aimed at determining the role of cytokines in the development of the neonatal immune system of swine. We will target characterization of intestinal and respiratory mucosal immune sites and their maturation under normal and stimulated conditions. These basic studies will enable us to assess neonatal immune system development as well as the effect of respiratory and parasitic infections on mucosal immune responses.
Cooperative studies with swine producers (PIC USA) will provide early weaned-pigs that will be treated with swine cytokines such as interleukin-12 (IL-12). It is hoped that early cytokine treatment will enable piglets exposed to shipping and translocation induced stress to better resist disease.

2. How serious is the problem? Why does it matter?

Pork producers have long known that there is a major loss in piglet

productivity as piglets move from the farrowing unit into nursery facilities at 3-4 weeks of age. This could be due to many infections caused by respiratory and intestinal pathogens or to the stress of the mixing of groups of animals. An additional concern is losses to the industry due to swine parasitism that erode consumer confidence in pork products due to the threat of zoonosis from trichinellosis, toxoplasmosis, and secondary bacterial contamination of tissues. Testing novel immunological controls for these infectious agents in neonates could result in improved disease resistance and productivity as well as prophylaxis and reduction in disease pathology.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401466 Year: 98 Project Number: 1265-32000-049-09 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 101 Animal Genome and Germplasm (50%); National Program Area 103 Animal Health (50%)
Studies on the basic mechanism of immunological control of infectious diseases of swine support development of vaccines against parasitic, viral and microbial infections that compromise animal health. These studies add basic and applied information concerning effective and sustainable methods to reduce disease associated production losses. The use of host immunity in an integrated control program will improve the efficacy of swine production systems, and result in overall healthier animals by reducing stress associated with infections.

4. What was your most significant accomplishment this past year?

The first grant objective, to determine the effect of age on the maturation of the intestinal and respiratory mucosal immune system and on functional responses, has been accomplished with a series of samples have been taken from pigs at PIC USA facilities between 1 and 40 days of age.
Protocols were standardized for isolation of cells from pulmonary alveolar lavage, and of different lymphocyte populations from peripheral blood, mesenteric lymph nodes, Peyer Patches, and intestinal sections.
Our collaborators at Schering Plough have expressed cloned swine IL-12, provided by us, using raccoon and swine virus vectors. Based on our biological assays they will select viral clones expressing the most cytokine protein. Using Western blots they have identified the expressed protein.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

This is the first full year for this project.

6. What do you expect to accomplish during the next year?

Reasonable quantities of expressed recombinant porcine IL-12 should be available in 1999 to begin to test its effect on neonatal pig mucosal immune system development. During the next year recombinant porcine IL-12 will be tested for activity in vitro. It will then be administered to neonatal swine to prophylactically control disease caused by viral, parasitic and microbial infections. These studies will demonstrate whether exogenous natural cytokine can substitute for the growth

Accession: 0401466 Year: 98 Project Number: 1265-32000-049-09 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.2 50% 3.2.1.4 50%
NATL PROG(S) 103 Animal Health 30%
108 Food Safety, (animal products) 70%

promoting and resistance enhancing properties of antibiotics. The success of this approach would have a major effect on minimizing the use of antibiotics in early pig weaning strategies. This approach is based primarily on our extended development of probes and reagents to study changes in host immune responses to parasitic infections.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

This collaborative project is funded through BRDC. As a result technology transfer is built into the project design. Provision of cloned cytokines to commercial partners is being pursued. We regularly update our collaborators on our progress and develop new project plans with their assistance.
The procedures developed with cytokine treatment of neonates should be widely applicable to pork production in the US. We will continue to update producers on our progress.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:
01.
SOLANO-AGUILAR, G.I. and LUNNEY, J.K. 1998. Mucosal Immune response in neonatal swine. Proceedings 15th International Pig Veterinary Society Congress, Birmingham, United Kingdom 2:283.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: PREVENTION & THERAPY FOR PROTOZOAN PARASITES AFFECTING FOOD ANIMALS, FOOD SAFETY, PUBLIC HEALTH

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The protozoan parasites Cryptosporidium parvum and Cyclospora cayetanensis are emerging as food- and water-borne pathogens worldwide. Both agents have been responsible for a significant number outbreaks of morbidity and mortality involving thousands to hundreds of thousands of persons in the United States during the 1990's. Cryptosporidium parvum is also a parasite of many other mammals and is especially prevalent in preweaned dairy and beef calves, causing morbidity and mortality with associated economic losses. A single infected calf can excrete tens of billions of infectious oocysts into the environment. Humans can acquire infection from the ingestion of less than 100 such oocysts in contaminated water or food items, resulting in several days of diarrhea and other flu-like symptoms. Immunocompromised persons are at special risk because pharmaceuticals are not available for treatment or prevention. Less is known about the natural history of Cyclospora, but outbreaks in the US and Canada have been traced to raspberries imported from Guatemala, pesto prepared and distributed by a gourmet grocery store chain, lettuce served on a cruise ship, and drinking water in a hospital dormitory. Currently, detection of these organisms in water or food items requires microscopy and special staining techniques, preferable performed by experienced personnel. The development of species specific, rapid, relatively simple, low cost detection assays would enhance our ability to identify and prevent agriculture-related outbreaks of these pathogens. Because livestock such as dairy and beef calves are so highly susceptible to infection with Cryptosporidium parvum and when infected, excrete billions of infectious particles (oocysts) that contaminate the environment and survive for months, it is

imperative to determine the sources of infection for these animals, the underlying basis for their susceptibility to infection, and to develop strategies to reduce such infection.

Avian coccidiosis, caused by several species of protozoan parasites belonging to the genus Eimeria, is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Current control strategies are losing their effectiveness. Therefore, the overall long-term goal of this project is to develop a novel immunological control strategy against coccidiosis. This is being accomplished by identifying various effector molecules secreted by cells

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

of the immune system (T-lymphocytes) that inhibit coccidia growth. Once identified, these molecules will be used to develop an immune based prevention strategy that is safe and logical and if successful, will reduce economic losses due to coccidiosis and possibly other diseases and thereby enhance poultry productivity.

2. How serious is the problem? Why does it matter?

In 1993, an outbreak of cryptosporidiosis occurred in Milwaukee, Wisconsin, ultimately involving more than 400,000 people, with nearly 100 deaths. The improper treatment of contaminated surface water at a drinking water treatment plant was responsible the outbreak. But this was only one of dozens of outbreaks related to drinking water contaminated with feces. Because clean drinking water starts with clean source water, protection of both surface and ground water from contamination with manure from animal feeding operations has become an issue of national importance. It is likely to affect decisions by such agencies as the USEPA and state environmental agencies by restricting animal farming practices in proximity to water. In addition, the impact of cryptosporidiosis on the animals themselves can be devastating, resulting in morbidity, poor growth, and even mortality, with associated economic loss. Outbreaks of Cyclospora cayetanensis in the US and Canada in 1996 through 1998 have involved thousands of clinical cases, imposed unnecessary financial hardship on U.S. strawberry growers, and ruined the marketing of Guatemalan raspberries in North America. It is obvious that this agent poses a continuing threat to the health of the citizens of the United States, and to the marketing of agricultural commodities. Coccidiosis is an economically important disease costing the poultry industry over $600 million in annual losses. Although drugs and live parasites are being used to control coccidiosis in chickens, problems associated with drug-resistance and antigenic variations in field strains of Eimeria parasites are increasing making these approaches less practical. Thus other approaches for coccidiosis control are urgently needed by the poultry industry.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (70%); National Program Area 108 Food Safety (30%)
Development of novel molecular biology-based assays, will permit accurate, rapid, low cost detection assays for Cryptosporidium parvum and Cyclospora parasites. Accurate detection methods for Cryptosporidium parvum will permit the rational development of land use policies, vaccination or medication protocols (when they become

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

available), food inspection, tracing of outbreaks or epidemics, and monitoring of drinking water to prevent waterborne outbreaks. Development of biologic/immunologic methods for reducing or preventing parasite infection such as cryptosporidiois in preweaned calves and coccidiosis in poultry will protect animal and human health by reducing the level of infectious parasites in the environment, thereby reducing the exposure level and the number of animals and persons that suffer morbidity and mortality. New immunological approaches will help overcome problems associated with drug-resistance and natural parasite antigenic variation.

4. What was your most significant accomplishment this past year?

A new molecular test, a nested polymerase chain reaction (PCR) assay involving a specific Cryptosporidium gene (Cp 11 discovered by ARS investigators) was developed and evaluated by detecting the presence of the parasite in oysters and water samples from the Chesapeake Bay, as well as in infected animal tissues. The assay will enable scientists and technicians in such places as hospitals, public health laboratories, and drinking water treatment plants to detect low numbers of parasites in these samples, levels below those ordinarily detectable by currently used techniques. A collaborative project with US Navy scientists used molecular biology techniques to examine Cyclospora cayetanensis from human patients. Gene sequence data is being generated to identify targets for diagnostic assays. ARS scientists produced recombinant Cryptosporidium parvum antigens and used antigens or DNA encoding these antigens to stimulate cows to produce colostrum with very high levels of antibody against this parasite. This colostrum will be tested to determine if it confers passive immunity against cryptosporidiosis. ARS scientitsts identified optimum doses of gamma irradiation for attenuating Cryptosporidium parvum oocysts to be used as a vaccine in calves against cryptosporidiosis. Through a CRADA, a recombinant immune reagent, the cytokine interleukin-12 (IL-12), was produced in sufficient quantity for testing in calves. Oysters were collected from 11 sites in Chesapeake Bay; some oysters from each site were found to have retained

Cryptosporidium oocysts filtered from the water. One of the most heavily contaminated sites was slightly downstream from two cattle farms. Scientific advances in disease and vaccine studies in poultry have been slow in the past due to limited progress in the development of various immunological reagents for this species. Last year we successfully produced two different recombinant biotherapeutics, immune cytokines, using genetic engineering technology. One of these cytokines, interferon-gamma, was able to promote the activation of a subpopulation of blood cells, the T lymphocytes. When chickens were treated with this factor it enhanced host immunity. Availability of these recombinant

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

chicken cytokine proteins will now enable scientists to investigate the feasibility of using these immune reagents in preventing economically important poultry diseases.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

ARS scientists identified antigens (proteins) of Cryptosporidium parvum useful for diagnostic purposes and cloned DNA sequences encoding these antigens. Patents were obtained and additional patents are pending. ARS scientists developed a nested polymerase chain reaction (PCR) assay for improved detection of Cryptosporidium parvum. By building upon an assay developed by other researchers in our group it is now possible to detect Cryptosporidium in shellfish, surface water, and animal tissues, where they would be undetected or under detected by conventional assays.
ARS scientists, in collaborative studies with the CDC, NOAA, and Johns Hopkins University scientists have demonstrated that shellfish will take up oocysts from water and thereby can serve as excellent indicators of water pollution. Studies, extended from the laboratory to the field, demonstrated contamination of oysters from Chesapeake Bay with Cryptosporidium oocysts in areas adjacent to cattle farms and in areas near waste water oufalls. Other shellfish (clams and mussels) were also found to filter and retain infectious Cryptosporidium oocysts from contaminated water. Migratory waterfowl were shown to be mechanical vectors of infectious Cryptosporidium parvum oocysts. Cryptosporidium parvum, the species that infects humans and livestock, was shown to be noninfectious for amphibians, reptiles, and birds, thereby eliminating them as ultimate sources of environmental contamination.
Time versus temperature charts were developed indicating how long oocysts can survive at environmental temperatures from -15 to 35 degrees Celsius (at 5 degree increments, and weekly intervals for 6 months). The effects of freezing and heating on oocyst survival was determined. Water and milk seeded with oocysts was passed through a commercial pasteurizer and it was found that oocysts were rendered noninfectious at 74 degrees Celsius in as little as 5 seconds.

ARS scientists determined that the reason newborn dairy calves and probably other young ruminants are so highly susceptible to infection with Cryptosporidium oocysts is because they lack sufficient numbers of mature lymphocytes at the site in the intestine where the arasites invade and develop. Studies were then initiated to stimulate movement of mature immune cells into the intestine.
ARS scientists identified the optimum dose of gamma irradiation that attenuated the infective stage of Cryptosporidium parvum (the oocyst stage) for future testing as a vaccine in calves. ARS scientists were the first to attempt vaccination of a domestic animal with DNA from a

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

parasite gene using gene gun injection. This study was done in pregnant sheep and resulted in production of colostrum with high levels of antibody against the parasite. We also used the gene gun to stimulate cows to produce colostrum with very high levels of antibody against Cryptosporidium that could be used for protection of calves against this infection.

Scientific advances in basic immunology in poultry have been slow in part due to lagging progress in the identification, purification, and production of immunological reagents for poultry. Our studies have shown that a certain type of immune cell, the T lymphocyte, which is located in the gut of coccidia-infected chickens, plays a major role in protecting the host against coccidia infection. Furthermore, our studies showed that this type of T lymphocytes produced soluble factors called cytokines following coccidia infection.

Last year, we showed that recombinant chicken gamma-interferon inhibited intracellular development of coccidia parasites in vitro. When tested in vivo this cytokine reduced parasite multiplication. Recombinant chicken interferon-gamma enhanced the infected host's immunity through the activation of specific cells, the macrophages. Therefore, better understanding of the ways that interferon-gamma activates macrophages and enhances host innate immunity against parasitic diseases will lead to the development of novel immunological control strategies against coccidiosis. Successful molecular cloning and biochemical and immunological characterization of interferon-gamma and another cytokine, interleukin-15 (IL-15), will enable comprehensive understanding of cytokine-mediated immune regulation and provide in-depth knowledge concerning the unique immunoenhancing properties of these cytokines. These studies in turn will lead to a logical immunological control strategies against poultry diseases.

ARS scientists identified optimum doses of gamma irradiation for attenuating major species of Eimeria and showed effectiveness against coccidiosis under three poultry rearing conditions: batteries, floor-pens, and field trials. They identified and cloned DNA sequences encoding immunodominant coccidial antigens and expressed these in fowlpox virus for developing a vaccine against coccidiosis.

6. What do you expect to accomplish during the next year?

Efforts will be made to develop an assay for "real time" molecular detection assays for quantitation of Cryptosporidium parvum infectious organisms. This assay will be based on the use of a fluorescing dye (fluorogenic probe) to specifically label infectious organisms. Once developed, this assay will be evaluated for its ability to aid control efforts at water treatment facilities, farm operations, and food safety. Investigation of the molecular biology of Cyclospora will continue, with

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

an emphasis on developing techniques to detect this organism in fresh fruits and vegetables. The effectiveness of hyper immune colostrum to reduce the severity of cryptosporidiosis will be tested in calves. The effectiveness of irradiation-attenuated Cryptosporidium parvum oocysts as a vaccine against cryptosporidiosis will be tested in mice. Molecular and immunological reagents will be developed for detecting oocysts of Cryptosporidium parvum in environmental water samples.
Studies will be conducted that will provide in-depth knowledge of the chicken cytokines, interferon-gamma and IL-15, and their immunobiology. Protocols for using these cytokines to enhance the vaccine responses with recombinant coccidia proteins will also be tested. Other vaccines, such as poultry fowlpox virus expressing proteins of Eimeria, will be tested for their ability to stimulate protection against coccidiosis. Attempts will be made to increase the expression of specific products from Eimeria for use in a vaccine.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

As participants in a collaborative project (Maryland Sea Grant) with investigators from the CDC, NOAA, and the Johns Hopkins University, oysters will continue to be harvested from various regions of the Chesapeake Bay and examined for the presence of Cryptosporidium, using the new nested PCR assay. This information is being utilized by environmental and public health scientists and administrators, state and federal agencies, to guide policies on food safety and agricultural management. As participants in a collaborative project [American Water Works Association (AWWA) grant], diagnostic methods for detecting oocysts of Cryptosporidium parvum in water will be transferred to water utilities via the AWWA. Immune-based therapies for preventing or ameliorating cryptosporidiosis in calves will be transferred to private industry via a CRADA.
A CRADA with the Nippon Zeon company has been established to develop a

recombinant fowl pox virus as a vector for carrying chicken cytokine genes for poultry disease control. We are also collaborating with other companies to develop various cell-mediated immunoassays to assess host immune response to various pathogens and DNA sequences encoding coccidial antigens have been transferred to several biotechnology companies for expression in vaccine vectors (e.g. fowlpox virus) for eventual testing in chickens against coccidiosis.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Accession: 0149613 Year: 98 Project Number: 1265-32000-050-00 D
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Making Coccidia Less Cocky, Agricultural Research, pp 20-21, January 1999.
Two Strategies for Protecting Poultry from Coccidiosis, Agricultural Research, p12, October 1996.
Video, 20 minutes, 1500 copies distributed worldwide, produced by USDA Working Group on Water Quality, entitled Cryptosporidiosis

PUBLICATIONS:

01.
LILLEHOJ, H.S. and CHOI, K.D. 1998. Recombinant chicken interferon-gamma inhibits Eimeria tenella development in vitro and reduces oocyst production and body weight ... acervulina. Avian Dis. 42:307-314.

02.
LILLEHOJ, H.S. and JAKOLEW, S. 1998. Mucosal gut immunity, pp. 105-108. IN: P. Pastoret, P. Griebel, H. Bazin, A. Govaert (eds.) Handbook of Vertebrate Immunology, Academic Press, New York, NY.

03.
LILLEHOJ, H.S. 1998. Role of intestinal lymphocytes and cytokines in avian coccidiosis. Proceeding of 73rd Annual American Society of Parasitologists Meeting, Kona, Hawaii, p. 48.

04.
WITHANAGE, G.S., SASAI, K., FUKATA, T., MIYAMOTO, T., BABA, E. and LILLEHOJ, H.S. 1998. Local immune responses in the oviducts of ... hens. Proc. 5th Int. Vet. Immunol. Symposium, Ludhihana, India p. 128.

05.
PARK, Y.H., HAHN, J.Y., OH, B.K., MOON, J.S., KOO, B.K., JOO, Y.S., SEO, K.S., LILLEHOJ, H.S. and DAVIS, W.C. 1998. Immunogenetic characterization of Korean ... antigens. Korean J. Vet. Res. 38:91-99.

06.
OH, J-Y., CHO, K.J., CHUNG, S.H., KIM, J.H. and LILLEHOJ, H.S. 1998.

Activation of macrophages by GLB, a protein-polysaccharides of the growing tips of Ganoderma Lucidum. Yakhak Hoeji 42:302-306.

07.
CHOI, K.D., LILLEHOJ, H.S., ZARLENGA, D.S. and NICHOLS, M. 1998. Kinetics of IFN-gamma gene expression ... infection. Proc. of 43rd Annual Meeting of American Asso. Vet. Parasitologists, Baltimore, MD p. 47.

08.
RAHMAY, S., SHERIFF, M., SASAI, K., LILLEHOJ, H. S., et al. 1998. Western blot analysis of chicken ... coccidia. In 34th annual scientific Seminar ... Diseases. Malasian Soc. of Parasitol. & Trop. Medicina. p. 22.

Publications: (Continued)

09.
JENKINS, M.C., TROUT, J. and FAYER, R. 1998. Development and application of an improved semiquantitative technique for detecting low-level Cryptosporidium parvum ... reaction. J. Parasitology. 84:182-186.

10.
JENKINS, M.C. 1998. Progress of developing a recombinant coccidiosis vaccine. International Journal of Parasitology. 28:1111-1119.

11.
LIDDELL, S., LALLY, N.C., JENKINS, M.C. and DUBEY, J.P. 1998. Isolation of the cDNA encoding a dense granule associated antigen (NCDG2) of Neospora caninum. Molecular and Biochemical Parasitology 93:153-158.

12.
GRACZYK, T.K., CRANFIELS, M.R. and FAYER, R. 1998. Oocysts of Cryptosporidium from snakes are not infectious to ducklings but retain viability after intestinal ... host. Vet. Parasitol. 77:13-40.

13.
GRACZYK, T.K., CRANFIELD, M. R., HELMER, P., FAYER, R. and BOSTWICK, E. F. 1998. Therapeutic efficacy of hyper immune bovine colostrum treatment against clinical and ... snakes. Vet. Parasitol. 74:123-132.

14.
PASZKO-KOLVA, C., SAWYER, T.K., PALMER, C.J., NERAD, T.A. and FAYER, R. 1998. Examination of microbial contaminants of emergency showers and eyewash stations. J. Industrial Microbiol. & Biotechnology 20:139-143.

15.
FAYER, R., GASBARRE, L., PASQUALI, P., CANALS, A., ALMERIA, S. and ZARLENGA, D. 1998. Cryptosporidium parvum infection in bovine neonates; Dynamic, clinical, and ... patterns. Int. J. Parasitol. 28:49-56.

16.
OLSON, E.J., EPPERSON, W.B., ZEMAN, D.H., FAYER, R. and HILDRETH, M.B. 1998. Effects of allicin-based product on cryptosporidiosis ... calves. Journal of the American Veterinary Medical Association 212:987-990.

17.
GRACZYK, T.K., FAYER, R., TROUT, J.M., LEWIS,E.J., .FARLEY, C.A., SULAIMAN, I. and LAL, A.A. 1998. Giardia sp. cysts and infectious ... canadensis. Applied and Environmental Microbiology 64:2736-2738.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400170 Year: 98 Project Number: 1265-32000-050-08 T
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: DEVELOPMENT OF CHICKEN HYBRIDOMAS AND OTHER IMMUNOLOGICAL REAGENTS FOR CHICKENS

Period Covered From: 04/97 To: 09/01

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Poultry industry is unable to adequately identify potential vaccine antigens of poultry pathogens. Mouse antibodies have been commonly used to identify immunogenic proteins of poultry pathogens. This methodology, however, has some limitation since mouse and chickens may recognize different types of foreign antigens. To overcome this problem, USDA developed a novel technology to produce chicken hybridomas. These hybridomas are immortalized chicken B cells and thus able to secrete chicken antibodies. Using this technique, chicken monoclonal antibodies detecting potential vaccine antigens of poultry pathogens can be produced in an unlimited amount, thus opening up the potential to clearly identify the most important poultry vaccine targets.

2. How serious is the problem? Why does it matter?

Over $500 million is being spent on poultry health product and vaccination. Much of this cost results from inadequate vaccination programs for viral, bacterial and parasitic diseases. Most poultry vaccines are attenuated pathogens and thus pose potential problems in the event of reverse mutation. Development of recombinant peptide or protein vaccines can overcome this problem. To develop recombinant protein vaccine for poultry, it is necessary to identify potential vaccine antigens that are immunogenic for poultry. For this, chicken hybridomas will be better than mouse hybridomas.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%).
Chicken monoclonal antibodies which detect potential vaccine antigens of poultry will lead to the development of effective recombinant vaccines and thus help to prevent many infectious diseases in birds.

4. What was your most significant accomplishment this past year?

B cell hybridomas which secrete monoclonal antibodies that can identify coccidia antigens have been developed.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400170 Year: 98 Project Number: 1265-32000-050-08 T
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Since chicken hybridomas secrete unlimited amount of chicken antibodies, commercial application of these monoclonal antibodies in immunodiagnostic assays will have a major impact on poultry industry.

6. What do you expect to accomplish during the next year?

Development of monoclonal antibody-based immunoassays for the identification of various poultry pathogens will be carried out.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Through this collaboration Agreement, USDA transferred chicken hybridoma technology to IDEXX for the development of a wide array of future poultry vaccine products.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400777 Year: 98 Project Number: 1265-32000-050-10 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: DEVELOPMENT OF FOWL POX VIRUS MEDIATED IMMUNE ENHANCEMENT AGAINST COCCIDIOSIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Coccidiosis is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Due to increasing incidence of drug-resistant field strains of coccidia, a novel approach is being undertaken. This CRADA was established with Nippon Zeon Company to develop a novel vaccination strategy against avian coccidiosis. Nippon Zeon Company developed fowl pox virus (FPV) vectors, which can carry foreign gene(s). Since the fowl pox viral strain that they are using as a delivery vector is the same virus strain as the field vaccine strain, this is an ideal vector for the poultry industry. The long-term goal this CRADA is to develop FPV recombinant vaccines, which carry both coccidia parasite genes and chicken immune stimulators, or cytokine, genes. If successful, this strategy will reduce economic losses due to coccidiosis and enhance poultry productivity.

2. How serious is the problem? Why does it matter?

Coccidiosis remains one of the economically most important diseases; it costs the poultry industry over $600 million annually in economic losses. Although drugs and live parasites are being used to control coccidiosis in chickens, problems associated with incidence of drug-resistance and antigenic variations in the field strains of Eimeria parasites are increasing making these approaches less practical. Thus novel approaches for coccidiosis control are urgently needed for the poultry industry.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%).
This approach will use immunological strategies to develop and test novel vaccines for chicken parasitic diseases. This will overcome problems associated with drug-resistance and antigenic variation of coccidia and reduce economic losses due to parasitic infection.

4. What was your most significant accomplishment this past year?

New vaccines made up of recombinant FPV vectors with coccidia gene and

Accession: 0400777 Year: 98 Project Number: 1265-32000-050-10 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

cytokine gene inserts have been developed. These constructs were stable and resulted in the expression of target proteins that was verified using various molecular and biochemical techniques.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Since recombinant FPV vectors carrying coccidia and/or chicken cytokine genes have been successfully produced, this will enable us to test their protective effect on challenge coccidia parasite infections. These studies will provide important information concerning the feasibility of using FPV vectors in poultry disease prevention programs.

6. What do you expect to accomplish during the next year?

A series of vaccination studies will be conducted next year to determine the efficacy of recombinant FPV vaccines and to obtain basic knowledge on the mechanisms of protective immunity induced by FPV vaccination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The CRADA with Nippon Zeon company was established to develop a recombinant FPV as a vector carrying coccidia and/or chicken cytokine genes for poultry disease control. Coccidia genes and chicken cytokine genes have been transferred to Nippon Zeon.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401222 Year: 98 Project Number: 1265-32000-050-12 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: DEVELOPMENT OF MUCOSAL IMMUNIZATION STRATEGY

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Coccidiosis is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Due to increasing incidence of drug-resistant field strains of coccidia, a novel approach is being undertaken. This CRADA was established with Novus International Company to evaluate a mucosal delivery system as a novel vaccination strategy against avian coccidiosis. The long-term goal of this CRADA is to develop a mucosal delivery injection system to vaccinate chickens with live coccidia vaccine.

2. How serious is the problem? Why does it matter?

Coccidiosis remains one of the economically most important poultry diseases, costing the industry over $600 million annually in economic losses. Although drugs and live parasites are being used to control coccidiosis in chickens, problems associated with incidence of drug-resistance and antigenic variations in the field strains of Eimeria parasites are increasingly making these approaches less practical. Thus novel approaches for coccidiosis control are urgently needed for the poultry industry.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%).
This approach will use a novel mucosal delivery system for vaccination of poultry against coccidiosis. It will reduce economic losses due to

parasitic infection.

4. What was your most significant accomplishment this past year?

Delivery conditions for optimal induction of protective immune responses against coccidiosis have been established using injection directly into the yolk sac of the developing chicken egg.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401222 Year: 98 Project Number: 1265-32000-050-12 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Feasibility of inducing protective immunity against coccidiosis using an injection machine which delivers coccidia vaccine into the chicken egg yolk will lead to a better vaccination strategy against coccidiosis. Since this study demonstrated that the intra-yolk sac delivery system induces as optimal a level of host protective immunity as the oral vaccination strategy, Novus International Company is planning to apply this technology to field trials.

6. What do you expect to accomplish during the next year?

A series of field trials will be conducted next year to determine the significance of this finding and to obtain basic knowledge on the mechanisms of protective immunity induced by intra-yolk sac vaccination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The CRADA with Novus International Company was established to develop a novel delivery vaccination strategy against intestinal diseases in poultry.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401540 Year: 98 Project Number: 1265-32000-050-13 T
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: DEVELOPMENT OF CYTOKINES AND IMMUNOLOGICAL REAGENTS FOR ENHANCED IMMUNITY IN CHICKENS

Period Covered From: 02/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The poultry industry is limited in its ability to assess host immune response to infections and vaccination in chickens due to the lack of availability of immunological assays. Since effective production of poultry is hindered by economic losses due to infections, the ability to adequately assess the immune status and vaccination efficacy against numerous viral, bacterial and parasitic disease will lead to reduced cost and increase poultry production. To address this problem, we established a Collaborative Agreement with Maine Biological Laboratories to develop immunological assays to assess the poultry immune system. Effective monitoring of the poultry immune status will reduce economic losses due to diseases and enhance poultry productivity.

2. How serious is the problem? Why does it matter?

Over $500 million is being spent on poultry health products and vaccination. Much of this cost results from inadequate monitoring of vaccination programs for viral, bacterial and parasitic diseases. Therefore, novel ways to assess poultry health are urgently needed to increase the efficiency of poultry production.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%).
Development of immunological assay systems which can adequately evaluate immune responses against major poultry diseases and to vaccination will

enhance poultry productivity.

4. What was your most significant accomplishment this past year?

Moue B cell hybridomas which secrete monoclonal antibodies that can identify various lymphocyte subpopulations and macrophages of poultry were originally developed at Beltsville. These cell lines have been recloned and transferred to Maine Biological Laboratories.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401540 Year: 98 Project Number: 1265-32000-050-13 T
Mode Code: 1265-20-00 STP Codes: 3.2.2.1 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Monoclonal antibodies which identify various lymphocytes and macrophages are crucial in the development of immunoassays for poultry health monitoring. Commercial application of these monoclonal antibodies in the identification of various cell subpopulations in chickens will enhance the ability of poultry industry to assess poultry health in their poultry flock.

6. What do you expect to accomplish during the next year?

Development of immunoassays for identification of chicken lymphocytes and macrophages will be carried out.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Through this collaborative Agreement, USDA transferred 8 different murine hybridomas which secrete monoclonal antibodies detecting chicken lymphocytes and macrophages to Maine Biological Laboratories. Chicken cell lines and techniques for hybridoma development were also transferred to Maine Biological Laboratories.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401860 Year: 98 Project Number: 1265-32000-050-14 T
Mode Code: 1265-20-00 STP Codes: 3.2.1.5 50% 3.2.2.2 50%
NATL PROG(S) 103 Animal Health 70%
108 Food Safety, (animal products) 30%

Title: INTERFERON-GAMMA MEDIATED ENHANCEMENT OF PROTECTIVE HOST IMMUNITY TO COCCIDIOSIS

Period Covered From: 09/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The long-term goal of this project is to obtain basic knowledge on the biology of important immune regulators, termed cytokines, in chicken vaccine and disease responses. One such cytokine is chicken interferon-gamma. This grant proposes to develop a novel immunological control strategy for control of the parasite induced disease, coccidiosis, based upon use of chicken cytokines. Avian coccidiosis is a major intestinal parasitic disease of poultry affecting nutrient absorption and optimal growth of poultry. Currently the poultry industry lacks suitable control strategies for this disease. A control strategy based upon use of lymphocyte-produced factors such as cytokines is safe and logical. If successful, this biological approach will reduce economic losses due to diseases and enhance poultry productivity.

2. How serious is the problem? Why does it matter?

Coccidiosis is an economically important disease, which costs the poultry industry over $600 million annually in economic losses. Although drugs and live parasite vaccines are being used to control coccidiosis in chickens, problems associated with incidence of drug-resistance and antigenic variations in the field strains of the Coccidia (Eimeria) parasites are increasingly making these approaches less practical. Thus novel approaches for coccidiosis control are urgently needed for the poultry industry.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program Area 103 Animal Health (100%)
Prevention of diseases requires novel immunological approaches. Use of new biotherapeutics, such as lymphocyte-secreted factors, or cytokines, will overcome problems associated with drug-resistance and antigenic variation of coccidian parasites.

4. What was your most significant accomplishment this past year?

This is a new NRI grant project, which was started in September, 1998.

ANNUAL RESEARCH PROGRESS REPORT
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5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

This is a new NRI grant, which started in September 1998.

6. What do you expect to accomplish during the next year?

Our studies have shown that intestinal cytotoxic T cells play a major role in host protection against coccidiosis. Furthermore, they indicate that a cytokine, which is secreted by activated T lymphocytes following coccidia infection inhibits an intracellular development of coccidian parasites in vitro and in vivo. One of these cytokines, interferon-gamma, has been shown to be the macrophage-activating factor and induces nitric oxide production by activated macrophages. Understanding the ways that interferon-gamma activates macrophages and enhances host innate immunity against parasitic diseases will enable scientists to devise novel immunological control strategies against coccidiosis. During the next year, studies will be performed to confirm these cytokine stimulated immune mechanisms in poultry. This will provide in-depth knowledge on chicken interferon-gamma immunobiology. The possibility of using these cytokines in enhancing the immunogenicity of recombinant coccidia proteins will also be tested using vaccine tests.
Scientific advances in basic immunology in poultry have been slow due to in part lagging progress in various immunological reagents for this species. Successful accomplishment in the molecular cloning and biochemical and immunological characterizations of avian cytokines will enable comprehensive understanding of cytokine-mediated immune regulation and provide in-depth knowledge concerning the unique immunoenhancing properties of these cytokines. These studies in turn will lead to a logical immunological control strategies against poultry diseases.

7. What technologies have been transferred and to whom? When is the

technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

Making Coccidia Less Cocky. 1999. Agricultural Research, January.

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